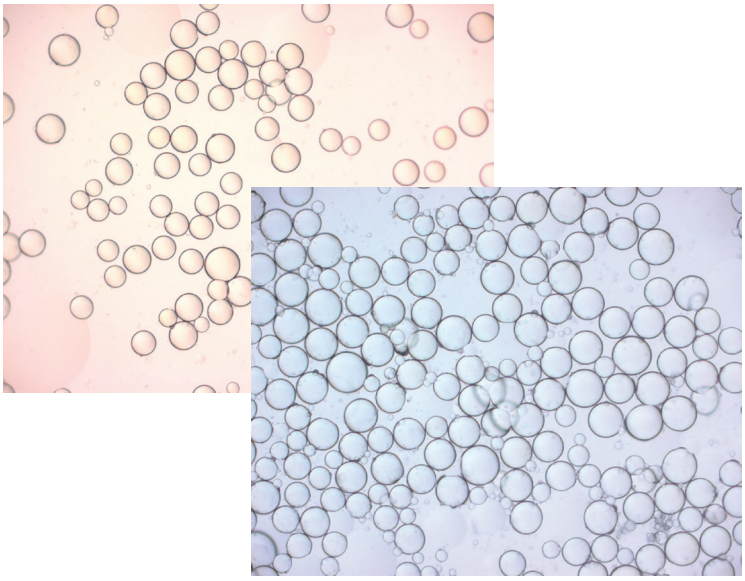
DISSERTATIONES SCHOLAE DOCTORALIS AD SANITATEM INVESTIGANDAM
UNIVERSITATIS HELSINKIENSIS

74/2016

SINI HEINONEN

Adipose Tissue Metabolism in Acquired Obesity



OBESITY RESEARCH UNIT
RESEARCH PROGRAMS UNIT
DIABETES AND OBESITY
FACULTY OF MEDICINE
DOCTORAL PROGRAMME IN BIOMEDICINE
UNIVERSITY OF HELSINKI

Obesity Research Unit,
Research Programs Unit, Diabetes and Obesity,
University of Helsinki
Finland

and
Faculty of Medicine
University of Helsinki

ADIPOSE TISSUE METABOLISM IN ACQUIRED OBESITY

Sini Heinonen

ACADEMIC DISSERTATION

To be publicly discussed,
with the permission of the Medical Faculty of the University of Helsinki
in Lecture Hall 1, Haartman Institute, Haartmaninkatu 3,
on November 25th 2016, at 12 o'clock noon

Helsinki, Finland 2016

Supervised by

Associate Professor Kirsi Pietiläinen
Obesity Research Unit

Professor Aila Rissanen
Obesity Research Unit

Research Programs Unit, Diabetes and Obesity,
University of Helsinki,
Helsinki, Finland

Reviewed by

Professor, MD Eero Mervaala
Pharmacology,
University of Helsinki, Helsinki, Finland

and

Professor Olavi Ukkola
Research Unit of Internal Medicine, Medical Research Center Oulu,
Oulu University Hospital
and University of Oulu, Oulu, Finland

Opponent

Associate Professor Gijs H. Goossens
NUTRIM School of Nutrition and
Translational Research in Metabolism
Department of Human Biology
Maastricht University Medical Centre
The Netherlands

Cover graphics Sini Heinonen:
Adipocytes floating on a microscope petri dish

ISBN: 978-951-51-2364-0 (paperback)
ISBN: 978-951-51-2365-7 (PDF)
ISSN: 2342-3161 (print) and
ISSN 2342-317X (online)

Dissertationes Scholae Doctoralis Ad Sanitatem Investigandam Universitatis
Helsinkiensis

Hansaprint, Helsinki 2016

"It is not the answers you give, but the questions you ask."
Voltaire

To my family and friends

CONTENTS

ABSTRACT

Obesity is a major health problem and is increasing rapidly both in developed and developing countries worldwide. Treatment of obesity is difficult, expensive and often fails. Obesity increases the likelihood for many diseases, such as type 2 diabetes, coronary heart disease, metabolic syndrome, hyperlipidemia and some types of cancer. We still lack a meaningful understanding of the factors behind this complex disease and therefore any proper means to battle it. Only lately, adipose tissue and especially its mitochondria have been recognized as important contributors to whole-body energy balance and the development of obesity.

This thesis investigates the biological pathways in adipose tissue that lead to the development of metabolic complications in early-onset obesity in young healthy twins. The aim was to study how acquired obesity affects adipose tissue and adipocyte function and how these link to whole body metabolism. The rare weight-discordant monozygotic (MZ) co-twin setting used in this study, is uniquely positioned to disentangle acquired and inherited metabolic pathways to disease in obesity. MZ twin pairs discordant for obesity enable controlling for genetic background, age, sex and early environmental influences. As MZ twins are fully identical at the level of genome sequence, the observed differences between co-twins can be assumed to be acquired. This is a major strength of our study regarding a polygenic and multifactorial trait as obesity.

Adipocyte hypertrophy in adipose tissue is one of the main features of obesity. The first study of the thesis investigated adipose tissue hypertrophy and hyperplasia in acquired obesity and its associations with whole body metabolism and gene expression pathways of the adipose tissue. We showed a high within-pair resemblance in adipocyte size and number suggesting that the adipocyte phenotype is genetic or due to shared environmental factors. Hypertrophy and low number of adipocytes in acquired obesity was related to metabolic dysfunction in obesity and associated with the disturbances in mitochondrial function and with increased cell death within the adipose tissue.

In the second study we investigated how transcriptional pathways of subcutaneous adipose tissue and the liver fat associate with “metabolically healthy obesity” – a phenomenon where some of the obese individuals stay free from the metabolic complications usually associated with weight gain. We showed for the first time in twins that the amount of liver fat is a key clinical determinant of metabolic health and that low liver fat associates with maintenance of high mitochondrial transcription and lack of inflammation in subcutaneous adipose tissue.

In further investigations we addressed mitochondrial biogenesis and oxidative metabolism in detail. The third and fourth studies concentrated on mitochondrial biogenesis in adipose tissue and in adipocytes, respectively. The novel findings in the third study were that obesity is related to reduced mitochondrial mass and oxidative metabolic activity in subcutaneous adipose tissue, both on the nuclear and on the mitochondrial transcription level, as well as to decreased protein levels in the OXPHOS system, especially OXPHOS complex subunits I and IV. The mitochondrial ‘dysfunction’ paralleled whole body insulin resistance and low-grade systemic inflammation. Remarkably, these changes were seen already in the early stages of acquired obesity. In the fourth study, we showed that the global downregulation of mitochondrial transcriptional signature in acquired obesity originates at least partly from the adipocyte cells of adipose tissue.

This research resulted in better understanding of the factors behind metabolic complications in acquired obesity. Development of obesity seems to associate with mitochondrial dysfunction in adipose tissue. The decreased function of mitochondria was evident at the level of both nuclear gene expression level and mitochondrial gene expression, as well as mitochondrial protein levels. These changes associated with metabolic disturbances of obesity. With rare obesity-discordant MZ twins we have been able to show that these changes are not genetic but result from acquired factors. However, as there was a remarkable similarity of adipocyte size and especially number between the co-twins, responses to obesity may have a partial genetic basis. With low capacity to adipocyte hypertrophy, excess fat may accumulate to liver and other tissues. Liver fat content is a clear determinant of metabolic health in acquired obesity. The results of my thesis as a whole suggest that obesity-associated metabolic disturbances might be halted by improving mitochondrial activity in adipose tissue.

TIIVISTELMÄ (in Finnish)

Lihavuus on nykyisin yksi merkittävimpiä kehittyneiden maiden kansanterveydellisiä ongelmia ja lisääntymässä nopeasti. Lihavuuden lääketieteellinen hoito on vaikeaa, kallista ja epäonnistuu usein. Lihavuus aiheuttaa metabolisia häiriöitä, kuten insuliiniresistenssiä, tyypin 2 diabetesta, sydänsairauksia, metabolista syndroomaa, ja veren kohonneita rasva-arvoja. Lihavuuden syistä ei ole edelleenkään kokonaisvaltaista ymmärrystä. Vasta viime vuosina on huomattu, että rasvakudos ja erityisesti sen mitokondrioiden toiminta ovat keskeisessä asemassa koko kehon aineenvaihdunnassa ja lihavuuden kehittymisessä.

Väitöskirjani käsittelee nuorten terveiden kaksosten rasvakudoksen varhaisia aineenvaihduntamuutoksia ja insuliiniresistenssiä hankitussa lihavuudessa. Mielenkiinnon kohteena on, kuinka lihavuus vaikuttaa rasvakudoksen ja rasvasolujen toimintaan ja koko kehon metaboliaan. Näitä muutoksia tutkitaan harvinaisten identtisten mutta eripainoisten kaksosten avulla. Tässä asetelmassa voidaan ainutlaatuisesti kontrolloida geneettinen tausta, ikä, sukupuoli ja aikaiset rasvakudokseen vaikuttavat ympäristötekijät ja näin erottaa hankitut ja periytyvät syyt lihavuuden aiheuttamien ongelmien synnyssä. Eroavaisuudet geeneiltään identtisten kaksosten välillä voidaan olettaa ympäristötekijöistä johtuviksi. Tarkoitukseen käytettävä suomalainen kaksosaineisto on kansainvälisesti merkittävä ja ainutlaatuinen. Kaksosasetelma on myös suuri vahvuus monitekijäisen sairauden, kuten lihavuuden, tutkimisessa.

Lihavuudessa erityisesti rasvakudoksen solujen koko kasvaa (hypertrofia). Ensimmäisessä väitöskirjan osatutkimuksessa selvitimme miten rasvasolujen suuri koko on yhteydessä rasvakudoksen geenien toimintaan ja koko kehon aineenvaihduntaan. Lihavuudessa havaittu rasvasolujen hypertrofia ja hypoplasia eli suuri koko ja pieni rasvasolumäärä olivat geneettisesti säädeltyjä ja yhdistyivät aineenvaihdunnan ongelmiin ja geenireitteihin, jotka liittyivät heikentyneeseen mitokondrioiden toimintaan ja lisääntyneeseen rasvakudoksen solukuolemaan.

Toisessa osatutkimuksessa selvitimme kehon rasvavarastojen ja rasvakudoksen geenireittien merkitystä ”metabolisesti terveillä” lihavilla, joille ei näytä ilmaantuvan lihavuuteen liittyviä metabolisia ongelmia. Havaitsimme ensimmäisinä, että maksan rasvamäärä voi olla merkki

metabolisten ongelmien kehittymisestä lihavuudessa. Mitokondrioiden aktiivisuus ja vähäinen rasvakudoksen tulehdus olivat yhteydessä matalaan maksan rasvapitoisuuteen ja ”metabolisesti terveeseen” lihavuus – ilmiöön.

Väitöskirjan kolmas ja neljäs osatyö käsittelivät tarkemmin rasvakudoksen ja rasvasolujen mitokondrioiden toiminnan häiriötä lihavuudessa ja mitokondrioiden toiminnan yhteyttä kehon aineenvaihduntamuutoksiin. Mitokondriot ovat solun energiatehtaita, jotka tuottavat solun tarvitseman energian ATP-yhdisteen muodossa. Mitokondriot tuottavat myös solun kasvuun, jakautumiseen ja erilaistumiseen tarvittavat yhdisteet ja niillä on oma pieni genominsa, jolla ne koodaavat osan omista rakenneosistaan. Kolmannessa työssämme näytimme ensimmäistä kertaa, että hankittu lihavuus vähentää mitokondrioiden massaa ja hengitysketjun toimintaa rasvakudoksessa. Mitokondrioiden toiminnan vähentyminen oli näkyvissä niin tuman koodaamien mitokondriaalisten geenien ja niitä säätelevien geenien ilmentymisenä, mitokondrion genomin kopiolukumäärän pienenemisenä, mitokondrion omien geenien ilmentymisen vähemenisenä kuin mitokondrion hengitysketjun proteiinien vähentyneinä määrinä. Näytimme myös ensimmäistä kertaa hengitysketjun komponenttien I ja IV vähentyneet määrät lihavuudessa. Mitokondrion huono toiminta yhdistyi aineenvaihdunnan ongelmiin, insuliiniresistenssiin ja tulehdusmerkkiaineiden suurentuneisiin pitoisuuksiin veressä. Sama mitokondriaalisen toiminnan väheneminen ja sen yhteys kehon huonontuneeseen rasva- ja sokeriaineenvaihduntaan oli nähtävissä väitöskirjan neljännessä osatyössä myös rasvasoluissa. Huomattavaa oli, että muutokset mitokondrioiden toiminnassa ja kehon metaboliassa olivat näkyvissä jo lihavuuden aikaisessa vaiheessa, nuorilla ja terveillä lihavilla kaksosilla.

Lihavuuden kehittyminen näyttää olevan yhteydessä rasvakudoksen mitokondrioiden toiminnan häiriintymiseen. Häiriintynyt toiminta näyttäytyy sekä tuman koodaamien mitokondrioon liittyvien geenien ilmentymisen kuin mitokondrion omien geenien toiminnan ja rakenneosasten määrän tasolla yhdistyen koko kehon insuliiniresistenssiin, tulehdukseen ja rasvakudoksen varhaisiin epäedullisiin aineenvaihdunnan muutoksiin. Harvinaisten identtisten mutta eripainoisten kaksosten avulla olemme voineet osoittaa, että muutokset geenien ilmenemisessä ja aineenvaihdunnassa johtuvat hankitusta lihavuudesta eivätkä geneettisistä tekijöistä. Geneettistä näyttää puolestaan olevan se, miten keho reagoi lihomiselle. Osa kaksosista kasvatti lihotessaan rasvasolun kokoa, toiset taas pystyivät lisäämään koon lisäksi rasvasolujensa määrää. Suuri rasvasolun koko ja vähäinen kapasiteetti lisätä niiden lukumäärää rasvakudoksessa näyttää aiheuttavan liian rasvan kertymistä maksaan ja muualle sisäelimiin. Maksan rasvapitoisuuden perusteella voidaan väitöskirjani mukaan mahdollisesti erottaa lihavat, jotka ovat alttiita lihomisen aiheuttamille komplikaatioille ja insuliiniresistenssille. Väitöskirjani tuloksien perusteella lihavuuteen yhdistyviä metabolisia häiriöitä voidaan mahdollisesti estää lisäämällä ja parantamalla mitokondrioiden toimintaa rasvakudoksessa.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by their Roman numerals.

I. **Heinonen S**, Saarinen L , Naukkarinen J , Rodríguez A, Frühbeck G, MD, PhD4, Hakkarainen A, Lundbom J , Lundbom N, Vuolteenaho K, Moilanen E, Arner P , Hautaniemi S, Suomalainen A , Kaprio J, Rissanen A , Pietiläinen KH; "Adipocyte Morphology and Implications for Metabolic Derangements in Obesity", *Int J Obes (Lond)*. 2014 Nov; 38(11):1423-31.

II. J. Naukkarinen , **S. Heinonen**, A. Hakkarainen, J. Lundbom, K. Vuolteenaho, A. Rodriguez, G. Frühbeck, P. Pajunen, T. Hyötyläinen, M. Orešič, E. Moilanen, A. Suomalainen, N. Lundbom, J. Kaprio, A. Rissanen, K.H. Pietiläinen; "Characterising metabolically healthy obesity in weight-discordant monozygotic twins." *Diabetologia*. 2014 Jan; 57 (1):167-76.

III. **Heinonen S**[#], Buzkova J[#], Muniandy M, Kaksonen R, Ollikainen M, Ismail K, Hakkarainen A, Lundbom J, Lundbom N, Vuolteenaho K, Moilanen E, Kaprio J, Rissanen A, Suomalainen A, Pietiläinen KH. "Impaired mitochondrial biogenesis in adipose tissue in acquired obesity". *Diabetes* 2015 Sep; 64(9): 3135-145.

IV. **Sini Heinonen**, Maheswary Muniandy, Jana Buzkova, Antti Hakkarainen, Jesse Lundbom, Nina Lundbom, Adil Mardinoglu, Amaia Rodriguez, Gema Frühbeck, Jaakko Kaprio, Aila Rissanen, Kirsi H. Pietiläinen "Mitochondria-related transcriptional signature is downregulated in adipocytes in obesity – a study of young healthy twins". *Diabetologia 2016 Oct 12, e-publication ahead of print*

# shared first authorship; authors contributed equally to the study

ABBREVIATIONS

AT	adipose tissue
ATP	adenosinetriphosphate
BCAA	branched-chain amino acid
BMI	body mass index
CI	complex I of the OXPHOS system
CII	complex II of the OXPHOS system
CIII	complex III of the OXPHOS system
CIRP	chronic inflammatory response pathway
CIV	complex IV of the OXPHOS system
CRP	C-reactive protein
CV	ATP-synthase (complex V of the OXPHOS system)
DEXA	dual energy x-ray absorptiometry
DNA	deoxyribonucleic acid
DZ	dizygotic
FAO	fatty acid β-oxidation
GCRMA	GeneChip robust multiarray averaging
GO	Gene ontology
HDL	high density lipoprotein particle
HOMA-index	homeostatic model assessment - an insulin resistance index
hs-CRP	High-sensitivity CRP
ICC	intra-class correlation
LDL	low density lipoprotein
Matsuda-index	an insulin sensitivity index
MHO	metabolically healthy obesity
MRI	magnetic resonance imaging
mRNA	messenger RNA
MRP	mitochondrial ribosomal protein
MRS	magnetic resonance spectroscopy
mtDNA	mitochondrial DNA
MZ	monozygotic
OXPHOS	oxidative phosphorylation system in the inner mitochondrial membrane
RNA	ribonucleic acid
rRNA	ribosomal RNA
SAT	subcutaneous adipose tissue
T2DM	type 2 diabetes mellitus
TCA	tricarboxylic acid cycle
VAT	visceral adipose tissue

1. INTRODUCTION

Obesity is a major health problem that is increasing rapidly all over the world. Obesity causes metabolic disturbances including insulin resistance and dyslipidemia. We still know very little of the molecular causes and consequences of obesity. The contribution of genes versus environmental factors to the development of metabolic complications in obesity is not known. Furthermore, not all obese individuals (10-40%) develop these metabolic disturbances (1, 2) and the underlying mechanisms are still unclear.

The enlarged adipocyte size or mitochondrial dysfunction in adipose tissue has been suggested to play a role in the metabolic problems of obesity (3-5). Mitochondria are needed for the anabolic functions of the cell and are essential for adipocyte differentiation (6). It has been proposed that adipose tissue dysfunction and the impairment in metabolic health in obesity (7, 8) would be due to the reduced oxygen consumption in obese adipose tissue (9), mediated by its mitochondria (10). Many cross-sectional studies (11-15), although not all (16), have shown that body composition with less visceral fat, and less ectopic fat accumulation in liver (16) and skeletal muscle (12, 16), would underlie a more metabolically healthy obese phenotype. The obese that accumulate metabolic problems have been suggested to have limited capacity of adipose tissue expandability, impaired adipocyte differentiation and thus limited capacity to store excess fat in adipose tissue, leading to accumulation of fat in internal organs and other ectopic sites (17, 18). Adipocyte hypertrophy, inflammation in adipose tissue and adipocyte dysfunction can be possible manifestations or mechanisms behind this. These functions may be genetically determined. The reason for the metabolic problems has however still remained unclear.

The TwinFat- study investigates the early gene expression differences in adipose tissue of young healthy monozygotic twin pairs discordant for obesity and the relation of these changes to metabolic alterations in glucose, insulin, lipids and other metabolic markers, body composition, inflammatory cytokines and mitochondrial biogenesis between the co-twins. The specific aim of this thesis was to study the gene expression pathways and the status of mitochondrial oxidative metabolism associated with obesity.

In this thesis, rare MZ obesity-discordant co-twins are used to disentangle acquired and genetic factors behind these pathways. Differences in investigated traits that correlate to weight-differences between the co-twins can be assumed to derive from acquired factors while extreme similarity between the twins is taken as evidence of genetic effect. We have previously shown significant differences in the global transcriptomic profiles of fat between the obese and non-obese identical co-twins. We have also shown that in obese individuals, mitochondrial DNA copy-number is downregulated, the expression of mitochondrial gene pathways reduced and inflammatory pathways up-regulated in acquired obesity (4, 19). This defective function of oxidative pathway components in mitochondria and the early phase of metabolic dysregulation in mitochondria seem to be key components associated with the development of metabolic complications in obesity and adipose tissue dysfunction. In addition to previous suggestions (4, 6, 20), the studies in this thesis have provided evidence that the metabolic problems of obesity begin with adipose tissue dysfunction including downregulation of mitochondrial biogenesis and function, and increased inflammation, conditions which are closely linked to fatty liver, insulin resistance and other metabolic complications.

This thesis describes biological pathways in adipose tissue that lead to the development of metabolic complications in obesity. The MZ twin co-twin setting, with twins of identical genome sequence, gave a unique possibility of detecting differences between the co-twins, which can be assumed to be acquired by obesity. Large adipocyte size and low adipocyte number in an individual associated with the metabolic unhealthy profile in obese co-twins. The extreme similarity between the co-twins in adipocyte number of the body suggested that adipocyte number and thus the expansion capacity of adipose tissue is genetically determined. The expandability of adipose tissue in turn relates to increase in liver fat amount and to the metabolic complications of obesity. Twins who had high liver fat content and large adipocytes were metabolically unhealthier than twins with low liver fat content and many small adipocytes in their adipose tissue. The increase in adipocyte volume, especially in the metabolically unhealthy twins was related to the downregulation of mitochondrial transcriptional pathways. The downregulation of mitochondria-related gene expression pathways, reduced mitochondrial DNA amount, downregulation of mitochondrial DNA transcripts and reduced levels of oxidative phosphorylation (OXPHOS) protein subunit levels in adipose tissue and adipocytes in obesity confirmed a widespread downregulation of mitochondrial biogenesis in obesity, associating with the metabolic derangements of acquired obesity.

2. REVIEW OF THE LITERATURE

2.1. Obesity – prevalence, complications and current treatment

Obesity is a major global health problem. In a large study conducted between 1980 and 2008, the prevalence of obesity doubled in every region of the world (21). In 2008, about 1.5 billion people were estimated to be overweight (BMI body mass index over 25 kg/m^2), 500 million of which were obese (BMI over 30 kg/m^2) (21). In 2012, 52% of European adults (OECD Health at a glance, Europe 2012) and 68% of adults in United States (22) could be characterized as overweight or obese. Childhood obesity has tripled in the last 30 years leading to health problems already at a young age (23). The rapid increase in the prevalence of obesity is mainly due to changing lifestyle factors; diet, eating behaviors (24, 25) and diminished exercise (26). Other factors linked to the development of obesity include genetic features (27), lack of sleep, psychosocial stress, viral and bacterial infections, fetal overnutrition, increasing gravida age (28) and gut microbiota (29).

Excess adiposity is associated with various disorders. Metabolic syndrome, insulin resistance, type 2 diabetes, hypertension, hyperlipidemias, atherosclerosis and cardiovascular diseases are major consequences of obesity (WHO: Obesity and overweight, Fact sheet N°311, Updated January 2015), (30). Metabolic syndrome often accompanies obesity and precedes type 2 diabetes and other complications of obesity including insulin resistance of the tissues, hypertension and dyslipidemia (31). Obesity-associated type 2 diabetes develops, when insulin secretion from pancreas is no longer sufficient for the needs of the insulin resistant tissues (32, 33). Obese individuals show an impaired blood vessel endothelial function (34, 35), linking obesity to the development of arterial stiffness and atherosclerosis. A chronic systemic and local low-grade inflammation is perceived in obesity and related to other undesirable metabolic changes (36). Stroke, congestive heart failure, pulmonary embolism, asthma, gallbladder disease (37) and gout are more common in obese than in lean persons. In addition, obesity increases the risk of breast, ovarian, endometrial, colorectal, esophageal, kidney, pancreatic and prostate cancers (WHO: Obesity and overweight, Fact sheet N°311, Updated January 2015) (30, 37). Excess weight gain can also lead to mechanical problems like obstructive sleep apnea (38), chronic pain and osteoarthritis (37). Psycho-social problems, increased probability of depression (39) and eating disorders may reduce the quality of life in obesity. Many of these factors are also linked to reduced life expectancy or premature death.

Treatment of obesity is difficult. Dietary and lifestyle modifications and exercise can be effective in the treatment, but changes in them can also be difficult to maintain. There is still a large need for an effective therapy to reverse the obese and diabetic phenotypes without side effects.

The diets generally used to combat obesity include low-caloric, low-fat, low-carbohydrate and very-low-calorie diets (40). However, when comparing all four diets no differences in the total weight loss after two years have been found (41). Generally, it is believed that the diet best suitable for the obese patient's taste is the best diet to that individual person. Exercise has proven important in maintaining weight loss achieved through caloric restriction (42), even though exercise alone seems to result in only very modest weight loss (43). Obesity surgery has proven beneficial and has helped patients with BMI $\geq$ 35 kg/m^2, but here too the results have sometimes been difficult to predict: while

some obese acheived significant weight loss, some did not lose enough weight and then there were some who continued to lose weight until anorexia.

Currently there are three drugs approved in Europe to treat obesity; orlistat, which inhibits pancreatic and gastric lipases that break triclycerides; glucagon-like peptide 1 receptor (GLP1R) agonist liraglutide, which increases insulin secretion and inhibits hyperglycaemia; and a combination of naltrexone–bupropion, which works by antagonizing the opioid system. Although these compounds have resulted in 3-12% weight loss, they also have various negative side effects and do not benefit all patients (44).

2.2. Fundamentals of adipose tissue and adipocytes

2.2.1. Adipose tissue in health and disease

Previously, white adipose tissue was thought to be a mere repository for the excess energy in the form of triglycerides. In recent decades, adipose tissue has been shown to be an important endocrine organ functioning in the regulation of whole-body metabolism, energy intake and fat storage and is thus also a major contributor in the metabolic consequences of obesity (45). Adipocyte lipid uptake and storage as triglycerides allows for expansion of adipose tissue and is an adaptive response to overnutrition, protecting other tissues from excess lipids. Beyond their role in nutrient handling and metabolism, adipocytes are highly active secretory cells producing hormones, adipokines and chemokines that contribute to immunity, inflammation, vascular growth and matrix remodeling (46). Adipose tissue is one of the largest organs in the body. Obesity causes excess fat accumulation into adipose tissue, testing the limits of its storage capacity. A general assumption is that when adipose tissue storing capacity is exceeded, fat starts to accumulate in internal organs, causing metabolic problems in the whole body (17, 47).

2.2.2. White adipose tissue distribution

White adipose tissue is divided into different depots, mainly subcutaneous and visceral adipose tissue (omental, mesenteric, retroperitoneal, pericardial and gonadal fat) as well as ectopic (intra-hepatic, intra-muscular, intra-pancreatic) fat, all of which have different characteristics and contributions to whole-body metabolism. Subcutaneous adipose tissue (SAT) is the largest body fat reserve and in total over 4-5 times larger than visceral adipose tissue (48, 49). Subcutaneous fat is divided between upper and lower body in a sex-specific manner (50) and both compartments contribute differently to the metabolic function of the body (51-53). Subcutaneous adipose tissue stores ~80-90% of the total body fat, mainly in the abdominal (around the waist), subscapular (on the upper back), gluteal and femoral (thigh) areas (48, 54). A smaller portion of the body fat (10-20% depending on sex and the individual) resides viscerally in the abdominal cavity (49).

2.2.3. Characteristics of white adipose tissue

White adipose tissue represents around 10%-20% of total body-weight in lean adults, but can achieve over 50% or even up to 70% in obese persons (55). Approximately 75% of the adipose tissue weight consists of lipids (56). The rest is an adipose tissue matrix, which includes collagen, blood vessels and lymphatic vessels as well as endothelial cells, smooth muscle cells, pericytes, immune cells (macrophages, lymphocytes and other inflammatory cells), fibroblast-like preadipocytes and adipocyte mesenchymal stem cells (ASCs) that together constitute the stromal vascular fraction (SVF) of the adipose tissue (53, 57). Adipose stem cells commit to preadipocytes – adipose precursor cells. (58-60). These cells are responsible for the maintenance, renewal and expansion potential of white adipose tissue in the normal and in the excess energy state (60). In response to energy needs, adipose tissue expands by increasing the cell size (hypertrophy) or the cell number (hyperplasia) in the tissue. 95% (61) of an adipocyte consists of triglycerides, which are stationed as one large lipid droplet inside the cell. The rest of an adipocyte is a small cytoplasm with all the cell organelles and an adipocyte cell membrane spanning around the cytoplasm and the lipid droplet. The fat stored is composed primarily of triglycerides and cholesteryl esters. Adipocyte mitochondria reside in the cytoplasm.

Figure 1: Adipocyte structure

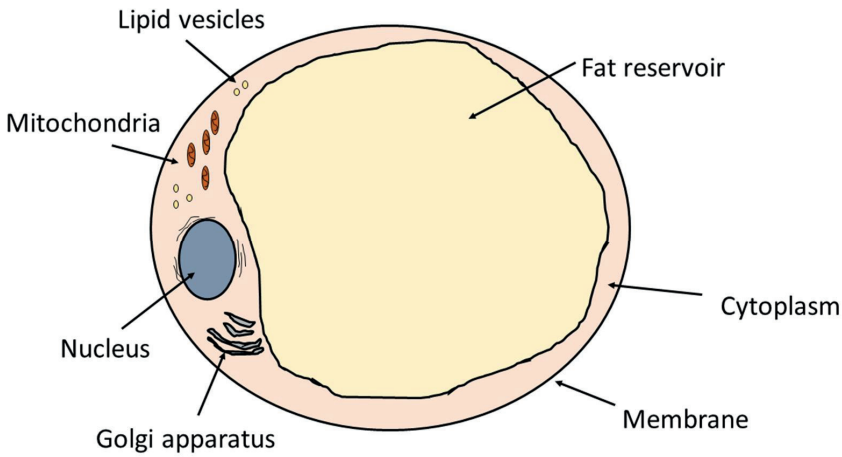


Figure 1: *Adipocyte structure. Triglycerides are stationed as one large lipid droplet inside the cell. Nucleus and other cell organelles are packed into a small space at the cell boundary. The cell membrane spans around the cytoplasm and the lipid droplet. Adipocyte mean size often ranges from 60 to 120 µm, but can be larger or smaller depending on the individual and the body fat mass.*

2.2.4. Other types of adipose tissue

White adipose tissue is the most prevalent and largest adipose tissue depot. However, also other types of adipose tissue exist. Brown adipocytes have different morphological and functional features than white adipocytes. They are smaller in size, with only ~50% of the cell volume occupied by lipids, which are divided into several small droplets inside the cell (62). Brown adipocytes in humans reside in small areas around the large blood vessels, especially in the supraclavicular region (62). Brown adipocytes do not store energy but dissipate it by producing heat. Their activity is regulated by the sympathetic nervous system (63). New research has also established a "beige" or "brite" adipocyte population that has characteristics of both white and brown adipocytes. These cells have thought to be in transition state between white and brown adipocytes and have the capacity to turn into either of them (62).

2.3. Fundamentals of mitochondria

2.3.1. Adipose tissue mitochondria in health and disease

The role of mitochondria in adipocytes was long neglected, perhaps because of their low abundance in these cells. Recent evidence has however shown that mitochondria in adipocytes are at the core of the energy metabolism of the cell, in healthy state actively controlling lipid turnover, producing ATP and substrates for cell metabolism as well as controlling the generation of new adipocytes, apoptosis and other essential functions of the cell (6, 64). Excess fat accumulation in obesity has been associated with mitochondrial dysfunction and impaired glucose and lipid metabolism in adipose tissue (6, 64, 65). In obese mice, a decrease in mitochondrial mass and function has been observed (66), as well as decrease in mitochondrial DNA (mtDNA) amount (67). Lower oxygen consumption rates in obese human preadipocytes compared with lean subjects have been recorded (68), and a reduced mitochondrial DNA copy-number in obese co-twins compared with their leaner counterparts (4). In healthy as well as in diseased state adipose tissue and its mitochondria have an important role in whole body metabolism.

2.3.2. Characteristics of mitochondria

Mitochondria in humans are organelles essential for the cell function, regulating many metabolic pathways by which chemical energy (from carbohydrates, lipids and proteins) is converted into energy-substrate ATP. Pyruvate oxidation, tricarboxylic acid (TCA) cycle, fatty acid β-oxidation and oxidative phosphorylation (OXPHOS) take place in mitochondria (69).

Mitochondria have two membranes; the outer membrane and the inner membrane, which is folded into cristae. These membranes divide mitochondria into the intermembrane space and an inner matrix subcompartments. The folded inner membrane houses the respiratory chain complexes (I-IV) and the ATP-synthase (complex V), which comprise the essential units of OXPHOS dependent energy production in the cell. The OXPHOS pathway consists of circa 90 protein subunits that are assembled into five complexes; Complex I (NADH dehydrogenase, 45 subunits), complex II

(succinate dehydrogenase, 4 subunits), complex III (cytochrome bc 1, 11 subunits), complex IV (cytochrome oxidase, COX, 13 subunits), and complex V (ATP synthase, 17 subunits) (70). In addition to the OXPHOS complexes, the respiratory chain has two mobile electron shuttles, ubiquinone (Coenzyme Q, CoQ), and cytochrome c (cyt c), a heme-containing small polypeptide.

Mitochondria possess their own multicopy genome, a 16.6 kb circular mitochondrial DNA (mtDNA) residing in the inner matrix. A special feature of this mtDNA is that it is inherited maternally. The mtDNA encodes two ribosomal RNAs (12S and 16S), 22 transfer RNAs, and 13 polypeptides (71). The two mtDNA-encoded rRNAs are constitutents of mitochondrial ribosomes; 12S RNA in the small 28S subunit and 16S RNA in the large 39S subunit of a mitochondrial ribosome (72, 73). In addition, 78 other structural proteins of mitochondrial ribosomes are encoded in the nucleus. The mitochondrial ribosomes are assembled in the mitochondria from nuclear-encoded proteins and the mitochondria-encoded RNAs. Mitochondrial ribosomes translate the mtDNA-encoded transcripts into the protein subunits of the OXPHOS complexes in the inner mitochondrial membrane (72).

The 13 mtDNA-encoded proteins are the core catalytic components of the OXPHOS complexes I, III, IV, and V (74). The remaining OXPHOS proteins are encoded in the nucleus. Complex II is solely nuclear-encoded. Also, about 1500 other mitochondrial proteins that are encoded in the nucleus —involving approximately 8% of all nuclear genes—act in mitochondrial maintenance, transcription and as structural components (75). These proteins are synthesized on cytoplasmic ribosomes and imported into mitochondria, where they are sorted into different compartments (76, 77). The nuclear-encoded subunits of the OXPHOS complexes translocate across the mitochondrial membrane (78) and are assembled into complexes with the mtDNA-encoded subunits. The OXPHOS complexes then bond together into larger “supercomplexes” (79) that facilitate the oxidative phosphorylation process and stabilize the complexes (80, 81). Mitochondrial ribosomes and the OXPHOS system are the only cellular entities that are encoded by both nuclear and mitochondrial genomes.

2.3.3. Functions of mitochondria

Mitochondria are required for both catabolic and anabolic functions in the cell (Figure 2). Energy production through an oxidative phosphorylation process (OXPHOS) is the best known function of mitochondria. Oxidative phosphorylation transforms the energy from digested carbohydrates, amino acids and fatty acids into ATP compounds for the cell to use. Oxidation of the energy substrates happens through the electron transport chain in the inner mitochondrial membrane. Complexes I, III and IV generate a potential difference across the inner mitochondrial membrane. Electrons derived from reducing agents of NADH and FADH2 from the TCA cycle or fatty-acid oxidation are shuttled through complexes I–IV to oxygen while forming water. The energy derived from this electron flux is used to pump protons (H+) from the mitochondrial matrix to the intermembrane space (82). This proton gradient is released by complex V (ATP-synthase) through a flow of electrons from NADH or FADH2 to oxygen and energy is generated by phosphorylation of ADP into ATP (83, 84). The complexes I, III and IV regulate the electron flux (85). The rate of ATP production is constrained by the cellular need for ATP. The generated membrane potential is used for the essential functions of mitochondria like protein import (76), fatty acid oxidation, generation of intermediary

metabolites, and to trigger changes to alter mitochondrial behavior. While working, complexes I and III generate reactive oxygen species (ROS) that can damage cellular proteins, nucleic acids and lipids (86). Disrupted electron flux leads both to energy depletion and to increased production of reactive oxygen species (ROS) (87).

In detail, complex I (NADH ubiquinone oxidoreductase) catalyzes the oxidation of NADH by Coenzyme Q (ubiquinone, CoQ). Complex I is the largest complex in the OXPHOS system (88). Seven of its subunits are encoded by the mitochondrial genome. Complex II is the smallest respiratory chain complex with four subunits, all encoded by nuclear genes. Complex III (ubiquinol–cytochrome c reductase) catalyzes the electron transfer from reduced coenzyme QH2, (ubiquinol) to cytochrome c. Complex III consists of 11 subunits (89) of which cytochrome b is encoded by the mtDNA. Complex IV (cytochrome c oxidase, COX), is the terminal component of mitochondrial respiratory chain and transfers electrons from reduced cytochrome c to molecular oxygen. COX is composed of 13 subunits (90), of which three are mtDNA-encoded. Complex V (ATP synthase) dissipates the proton electrochemical gradient generated by the respiratory chain across the inner mitochondrial membrane to produce ATP. It comprises of a membrane-bound cylindrical rotor-like structure, the F0 particle and a matrix-facing F1 particle, the catalytic ATP synthase domain (83). All five subunits of F1 and all except two subunits of F0 are nuclear-encoded.

The mitochondrial tricarboxylic acid cycle (TCA) /citric acid cycle (Figure 3) in the mitochondrial matrix generates the reducing equivalents NADH and FADH2, which donate electrons to the electron transport chain, and energy compounds (ATP/GTP) for the cell to use. As its intermediate products TCA creates substrates that are used for carbon skeletons of many non-essential amino acids, needed for the synthesis of hemoglobin, myoglobin and various cytochromes, used for fatty-acid synthesis and production of cholesterol (Acetyl-CoA), and exported from mitochondria to be used in adipogenesis (citrate) in adipose tissue and gluconeogenesis in liver (oxaloacetate) (91). Breakdown of excess amino acids and conversion of excess nitrogen into urea take place in mitochondria. Fatty-acid β-oxidation in mitochondria converts dietary long chain fatty-acids to Acetyl-CoA to be used in energy production (92). Mitochondria control many biosynthetic pathways like steroid synthesis (93), Fe-S synthesis (94) and heme synthesis (95). They have an important role in programmed cell death, also known as apoptosis (96). Mitochondrial also store calcium, functioning as the calcium buffer of the cell, controlling cellular calcium signals and Ca2+ flux from the plasma membrane and the endoplasmic reticulum (97).

Figure 2: Mitochondrial functions in adipose tissue

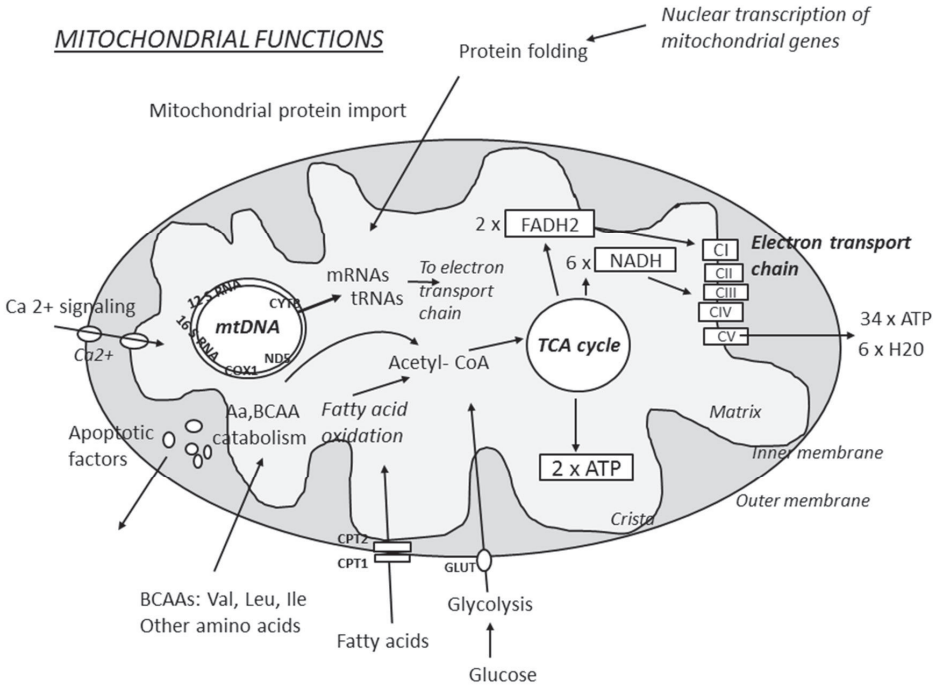


Figure 2: *Mitochondria have various functions in the cell. Amino acid (Aa) and branched-chain amino acid (BCAA) catabolism and long-chain fatty acid oxidation occur in the mitochondrial matrix. These together with cytoplasmic glycolysis produce Acetyl-CoA and other substrates for the TCA cycle. TCA cycle generates energy for the cell in the form of ATP as well as reducing equivalents (NADH, FADH2) for the electron transport chain. The electron transport chain in the inner mitochondrial matrix transfers electrons from one complex to another and finally to complex IV, which reduces oxygen to water. The transfer of electrons is coupled to the pumping of protons into the intermembrane space by complexes I, III and IV. The resulting proton gradient is harnessed by complex V, whereby the force driven by the flow of protons into the matrix is used to phosphylate ADP to ATP. Mitochondrial DNA (mtDNA) transcription produces mRNAs and rRNAs, which are structural parts of mitochondrial ribosomes and the OXPHOS complexes. A large number of mitochondrial structural and functional proteins are however encoded in the nucleus and transported into mitochondria. Mitochondria control Ca2+ signaling in the cell and cell apoptosis. CI-CV = complexes 1-5 of oxidative phosphorylation (OXPHOS).*

Figure 3: TCA cycle

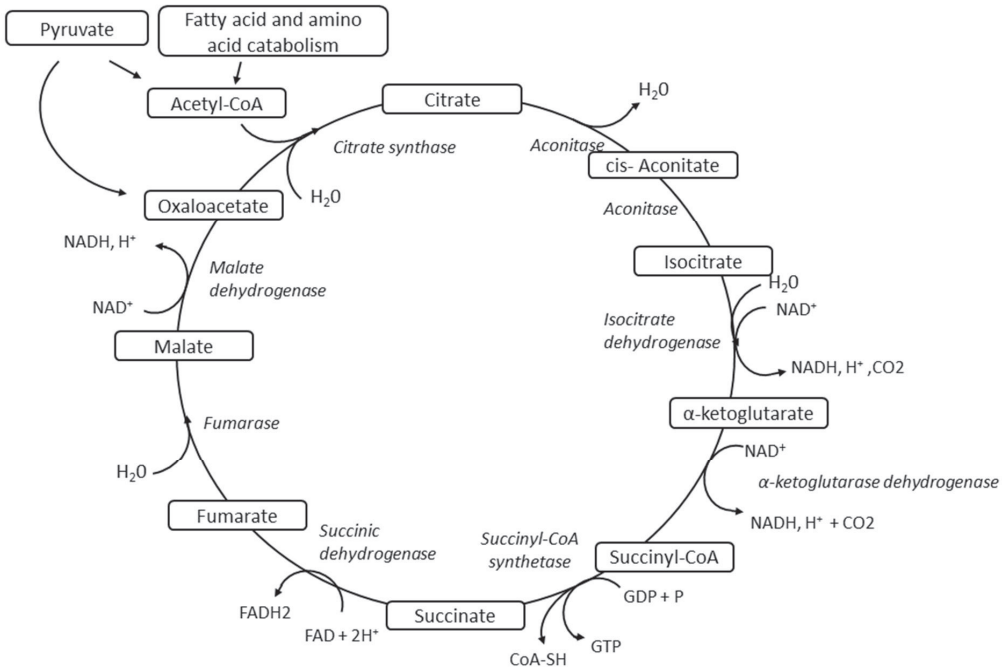


Figure 3: *Tricarboxylic acid (TCA) cycle. Energy substrates converted into Acetyl-CoA enter the TCA cycle. In intermediate steps, Acetyl-CoA is converted into different substrates and in due course, reducing equivalents (NADH, FADH2), CO2 and energy (ATP, GTP) are generated.*

2.3.4. Regulation of mitochondrial number and function in the cell

Mitochondria constantly remodel themselves by biogenesis, fusion and fission as well as by travelling in the cell (98, 99). The cell regulates the function of its mitochondria based on its energy needs. Pancreatic β-cells and muscle cells in a nutrient-rich environment have morphologically fragmented mitochondria, but in starvation their mitochondria elongate (100, 101). In muscle studies starvation increases mitochondrial ATP synthesis capacity (100) and increase in mitochondrial mass and OXPHOS activity are observed after endurance exercise (102). Adipose tissue in nutrient excess is suggested to be characterized by a decrease in mitochondrial function, biogenesis or content (103-105). It has, however, remained controversial if there is a reduction in all or in only some of these features.

The energy status information in the cell is transmitted through NAD+:NADH ratio, the AMP:ATP ratio and Acetyl-CoA levels, which sense the signals of mitochondrial activity. Two key cellular energy sensors are the AMP-activated protein kinase (AMPK) and a NAD+-dependent deacetylase

SIRT1 (106, 107). AMPK enhances oxidative phosphorylation and other catabolic functions of the cell, while suppressing cell proliferation and growth (108). SIRT1 regulates mitochondrial mass, nutrient oxidation, and ATP production. Together, they act through the transcription cofactor, peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α). By changing the function, morphology, number, organization and distribution of the mitochondria within the cell, the cell adapts to different energy demands, cell growth, cell death and differentiation (109).

Mitochondrial biogenesis needs an orchestrated induction of transcriptional regulators, activated by energy demands (110). PGC-1α is one of the main inducers of mitochondrial oxidative metabolism (111). PGC-1α interacts with various mitochondria-related transcription factors and hormone receptors (112) to fine-tune mitochondrial metabolism, and has a major role both in adipogenesis and mitochondrial biogenesis. The activity of PGC-1α is regulated by acetylation. In a normal state, PGC-1α is acetylated and silenced. However, in nutrient excess, SIRT1 activates PGC-1α and mitochondrial activity is enhanced (113). PGC-1α regulates the functions of TFAM, which controls the transcription of mitochondrial proteins (114) and NRF-1, which is needed in the induction of mitochondrial biogenesis (115). PGC-1α also targets estrogen-related receptor α (ERRα) and GABPα to regulate the OXPHOS complexes including cytochrome c and ATP synthase (116, 117). The transcription factor Forkhead box O 1, FOXO1, is another important mitochondrial regulator, which enhances the expression of genes involved in mitochondrial lipid oxidation and oxidative stress protection (118).

2.4. Functions of adipose tissue and its mitochondria

2.4.1. Adipogenesis and adipocyte turnover in adipose tissue

Adipose tissue enlarges by increasing adipocyte size (adipocyte hypertrophy), by recruitment of new adipocyte cells in adipogenesis (hyperplasia), or both. Increase in the number of adipocytes (hyperplasia) or turnover of the old cells is thought to happen throughout life by preadipocyte differentiation (119, 120). A study on adipocyte turnover in white adipose tissue used C14 detection to suggest that approximately 10% of the body's fat cells are regenerated each year at all adult ages and levels of body mass index and that the formation of adipocytes is a lifelong and a regulated process (120). This study compared the levels of C14 in atmosphere versus in adipose tissue in persons with different BMIs, born before and after the nuclear bomb tests of 1955, which caused a rise in C14 in the atmosphere. The levels of C14 have since decreased due to diffusion from the atmosphere. Comparing the levels of atmospheric C14 at the time point when the persons were born to the current C14 levels in their adipose tissue gave indication how many cells had been renewed since the individuals were born. The number of adipocytes in adipose tissue seem to be largely determined by the ability of the limited number of preadipocytes to undergo the differentiation process as well as the amount of mesenchymal stem cells that are available for differentiation into new preadipocytes when necessary (121).

Adipocytes are believed to originate from mesenchymal stem cells, but the early steps leading the mesenchymal stem cells to commit to adipocyte lineage and form the adipocyte precursor cells,

preadipocytes, have remained unknown (122). The cells that are capable of differentiating into full mature adipocytes (adipose stem cells and preadipocytes) reside in the adipose stroma (59). The adipocyte stem cells may originate from perivascular cells, at least partly, through transforming into mural cells and then to a progenitor population (123, 124). However, not all adipose tissue originates from the mesoderm. Mouse embryonic stem cells have been shown to be able to commit to adipocyte lineage (125). Some recent studies in humans raise the possibility that hematopoietic stem cells from bone-marrow (126) might give rise to a certain subset of adipocytes and also neuroepithelial cells seem to have the capacity of generating adipocytes (127).

Adipogenesis means the differentiation of mesenchymal stem cells into fibroblast-like preadipocytes, which then differentiate into mature lipid-rich adipocytes (128). In the first step of the differentiation, the pluripotent stem cell commits to unipotent preadipocyte lineage, where it has lost the capacity to differentiate into other cell types. In the second step called terminal differentiation, preadipocytes undergo marked changes in their gene expression and morphology and acquire the phenotype and characteristics of mature adipocytes. When adipocytes are fully mature, they are incapable of differentiation and of cell division.

The maturation of stem cells into preadipocytes is dependent on the expression of extracellular factors like transforming growth factor β (TGFβ), the bone morphogenetic proteins (BMPs), insulin-like growth factor 1 (IGF1), interleukin 17 (IL17), activin, fibroblast growth factor 1 (FGF1), and FGF2 (129). Silencing of Wnt signaling (130) and Hedgehog signaling (131) pathways are important for the differentiation to proceed.

Changes from preadipocytes to adipocytes include becoming sensitive to insulin, expressing fatty-acid-binding protein (422/aP2), lipoprotein lipase (LPL), adipsin and GLUT4 on the cell surfaces and beginning the uptake of glucose, induced by insulin (132). This terminal differentiation step is dependent primarily on the transcription factors PPARγ (115, 133), its activator PGC-1α, as well as transcription factor C/EBPα (134), sterol regulatory element-binding protein-1 (SREBP1) (135), mTORC (136) and the forkhead transcription factor FOXO1 (137), the latter of which may link the effects of insulin to the differentiation process. Low levels of these factors are found in preadipocytes, but during adipocyte differentiation their expression increases (133). PGC-1α and mTORC1 (138), enhance adipogenesis through their effect on PPARγ, but also independently by enhancing insulin sensitivity of the differentiating preadipocytes (139).

Mitochondria have been recognized as essential effectors in adipocyte differentiation and adipogenesis (6). The first microscopic observations on changes in mitochondrial number and morphology during adipogenesis were made already in 1962 (140, 141). The increased oxygen consumption of preadipocytes during the differentiation process was thought to be evidence of increased mitochondrial biogenesis (66, 142, 143). A 20- to 30-fold increase in the concentration of mitochondrial proteins during adipose differentiation and marked changes in mitochondrial morphology have been recorded (66).

Coordination between adipogenesis and mitochondrial biogenesis is supported by the fact that the same transcription factors are needed in both processes. PPARγ, C/EBPα, CREB, (133), estrogen-related receptor α (ERRα) (144) and PGC-1α are all major regulators of mitochondrial biogenesis,

adipogenesis and body energy balance (145). The link between mitochondrial biogenesis and adipogenesis was demonstrated by treatment of adipocytes with rosiglitazone, a PPARγ agonist, which caused marked alterations in mitochondrial morphology and density (142), suggesting that the nuclear-encoded mitochondria-related genes are under major control of PPARγ, a strong inducer of adipogenesis (133).

Mitochondria, or their dysfunction, may also inhibit adipogenesis. Mitochondrial reactive oxygen species (ROS) production by complexes I and the ATP-synthase inhibit proliferation of 3T3-L1 mouse preadipocytes (146) and prevent adipocyte differentiation in mice (147). However, ROS levels are also believed to play an important role in signalling during the differentiation (148). Thus it seems that the amount and the duration of ROS effect are critical in the initiation of normal versus pathological responses in adipocyte differentiation. Normal mitochondrial function is needed to enable and drive adipogenesis in adipose tissue and defects in this might thus affect the differentiation capacity and possibly the expansion potential of the tissue.

Figure 4: Adipogenesis

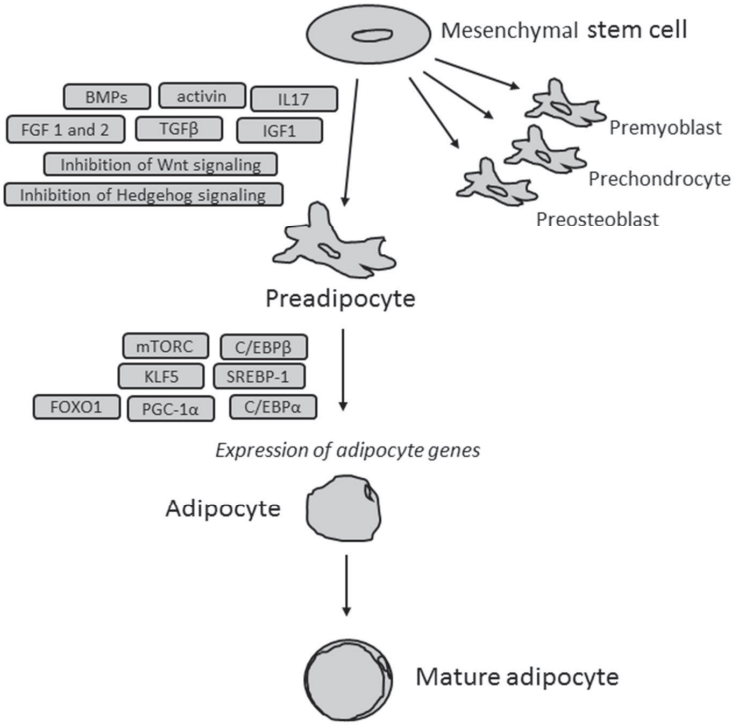


Figure 4: *Mesenchymal stem cells differentiate into preadipocytes. This is initiated and enhanced by IL17, FGF1 and 2, TGFβ, bone morphogenic proteins (BMPs) and activin. Silencing of Wnt signaling and Hedgehog signaling is needed. Preadipocytes differentiate into lipid-laden*

adipocytes. Here, PGC-1α, FOXO1, C/EBPα, KLF5, SREBP-1, mTORC and C/EBPβ function as main regulators and enhancers.

2.4.2. Lipogenesis and lipolysis

The key function of adipose tissue is to, depending on the body energy state, expand and store excess energy in the form of triglycerides (lipogenesis), or release them into circulation and other tissues as free fatty acids (FFA) (lipolysis) when needed. 1000g of fat contains 800g of triglycerides and circa 7000 kcal of energy. An average life span of an adipocyte in subcutaneous white fat is 9.5 years, during which time its lipid storage is replaced six times on average (120). In obese state there is increased lipid storage and decreased lipid removal compared to lean persons meaning an overall increased lipid turnover rate (120).

Lipogenesis refers to the FFA or glucose uptake into adipocytes and their subsequent synthesis into triglycerides (TAG's) (149). Activation of adipose tissue lipoprotein lipase (LPL) is crucial for lipogenesis. LPL is synthesized by adipocytes and transferred into the capillary endothelial lumen, where it liberates FFA's from circulating chylomicron particles. The FFAs diffuse through capillary endothelium, where they are taken up into adipocytes by fatty acid translocases (150). Two enzymes, Acetyl-CoA carboxylase and fatty-acid synthase, use Acetyl-CoA and malonyl-CoA from glucose, pyruvate or other carbon precursors to generate palmitate. This palmitate and the FFAs can then be modified to produce multiple lipid species.

Mitochondria are essential in generating the intermediary metabolites needed in lipogenesis (6). The generation of Acetyl-CoA for fatty acid activation and synthesis before their esterification into triglycerides requires active mitochondria. Interventions that reduce mitochondrial ATP-generation (151) or the uncoupling of the electron transport chain (152) are shown to decrease the rate of lipogenesis in mice studies and thus fatty acid deposition into adipocytes. The activation of medium-sized fatty acids for lipogenesis happens in the mitochondrial matrix. Long-chain fatty acids are activated both in mitochondrial matrix and endoplasmic reticulum. Mitochondria are also an important site for phospholipid synthesis and thus control the structure of the cell membrane (153).

Insulin is the key regulator of fatty acid uptake and metabolism in adipocytes. Insulin increases the recruitment of glucose transporters (GLUT4) to the adipocyte membrane and activates lipogenic and glycolytic enzymes. Insulin stimulates PPARγ, an important lipogenic and adipogenic enhancer. In the long-term, insulin promotes lipogenesis by enhancing the transcription of lipogenic genes trough regulatory factor SREBP-1 (149). SREBPs are transcription factors that coordinate fatty acid and cholesterol metabolism and mediate the lipogenic action of hormones and nutrients. In addition to insulin, lipogenesis is promoted by acyl-stimulating protein ASP and prostaglandins whereas growth hormone and leptin inhibit lipogenesis (149).

During fasting, adipose tissue releases FFA by lipolysis. Triglyceroles are hydrolyzed into di- and monoglyceroles and glycerol, and transported from adipose tissue into circulation for liver, muscle and other tissues. Lipolysis requires structural reorganization in the lipid droplets to enable the

hydrophilic lipases to access the hydrophobic lipid inside the droplets. Perilipin moves away from the droplet surface to allow the enzymes in (154). The hormone-sensitive lipase (HSL) and its regulation is the rate-limiting step of lipolysis (155). HSL liberates two fatty acids from the triglycerol backbone by hydrolysis. Adipose triglyceride lipase (ATGL) (156) has recently been characterized as another lipase in this initial hydrolysis step. 2-monoacylglycerol (MAG) lipase then catalyzes the liberation of the remaining fatty acid and glycerol. FFAs can be transported out of the cell or re-esterified back to new triglycerides. The glycerol produced in adipose tissue however cannot be used as energy source there, because adipose tissue has only a limited activity of glycerol kinase. Glycerol from adipose tissue is thus transported to the liver for gluconeogenesis.

The regulation of lipolysis is sensitive to the energy status of the adipocyte (the ATP levels of the cell), maintained by its mitochondria. Lipolysis and mitochondrial ATP synthesis were shown to be coupled already in 1975, when inhibitors of the electron transport chain function shut down catecholamine-induced lipolysis (157). Mitochondrial energy sensor cyclic adenosine monophosphate (cAMP) works as the main second messenger of ATP levels in the cell during lipolysis. Its high levels enhance the protein kinase A (PKA) activity and lipolysis. Catecholamines and glucagon stimulate lipolysis in the fasting state, and insulin and AMPK inhibit it in energy excess (158).

Only a small fraction of the FFAs released as a result of adipose tissue lipolysis are oxidized for energy needs. Instead, the majority are re-esterified back into triglycerides in adipose and other tissues. In adipose tissue the FFA re-esterification by de novo lipogenesis is generally low (159) but occurs during low glucose supply or fasting (160, 161). The amount of re-esterification, independent of the metabolic state, is up to 75% and during fasting, adipose tissue accounts for 20-30% and liver for circa 50% of this amount (161).

2.4.3. Fatty acid oxidation

Inside the adipocyte, FFAs are esterified, metabolized or β-oxidized in mitochondria. In fatty acid oxidation, the long-chain fatty-acids are transported into the mitochondrial matrix. Carnitine palmitoyltransferases (CPTs; CPT1, CACT, CPT2) (162) facilitate this step. As the result of the β-oxidation Acetyl-CoA is generated and enters the tricarboxylic acid (TCA) cycle for cellular respiration and ATP production. Malonyl-CoA from glucose metabolism and lipogenesis regulates fatty acid oxidation by inhibiting the function of CPT1. AMP-activated protein kinase AMPK inhibits ATP-consuming processes and activates catabolic pathways. AMPK thus increases β-oxidation by decreasing the flux of substrates in the anabolic pathway of fatty acid synthesis (163). In consequence, Malonyl-CoA levels in the cell drop leading to an increase in CPT1 activity and to fatty acid β-oxidation.

2.4.4. BCAA oxidation

Adipose tissue is nowadays recognized as one of the main sites of mitochondrial branched-chain amino acid (BCAAs; including leucine, isoleucine and valine - three of the nine essential amino acids) catabolism (164, 165). In contrast to the other 17 amino acids which are metabolized in the liver, BCAA's are only poorly metabolized there. In circulation, they continue to peripheral tissues and act as nutrient signals of amino acid intake, regulate protein synthesis and degradation, impact insulin secretion and act in the neural control of food intake and energy balance (166, 167). Adipose tissue was already earlier suggested to be the main site of excess BCAA conversion into fat (168) and estimated second only to skeletal muscle in metabolizing BCAAs, the metabolism in liver being 6-7 fold less than in these two tissues (164, 169). However, the two first enzymes of the BCAA oxidation pathway across many tissues, BCAT2 and BCKD, were found to be less active in adipose tissue than in other tissue depots (170). Thus, the quantitative role of adipose tissue in BCAA catabolism remained unclear. In 2010 Herman et al demonstrated that adipose tissue is an important site of BCAA catabolism and indeed has the capacity to regulate levels of circulating BCAAs *in vivo* (165).

The first step in BCAA catabolism is a reversible transamination reaction by branched-chain amino-acid aminotransferase (BCAT) to form their respective α-ketoacids. There are two mammalian BCAT enzymes: a cytosolic (BCATc, BCAT1) and a mitochondrial (BCATm, BCAT2) isoenzyme (171). The second step in BCAA metabolism is an irreversible oxidative decarboxylation reaction of the branched-chain α-keto acids (BCKAs) and catalyzed by the mitochondrial branched-chain α-ketoacid dehydrogenase (BCKD) complex. After additional steps the metabolic pathway produces substrates that are used in the TCA cycle in mitochondria.

When mitochondrial mass is elevated in adipogenesis, the expression of enzymes involved in BCAA catabolism increases (172). Mitochondrial metabolism of BCAAs stimulates adipocyte growth and differentiation by activating rapamycin signaling (173) and a decrease in BCAA catabolism reduces the substrates for this pathway. While adipose tissue BCAA catabolism decreases, plasma BCAA levels rise (165). Until now, it has not been known if decreased catabolism of BCAAs could be one factor behind impaired adipocyte differentiation.

Figure 5: BCAA catabolism

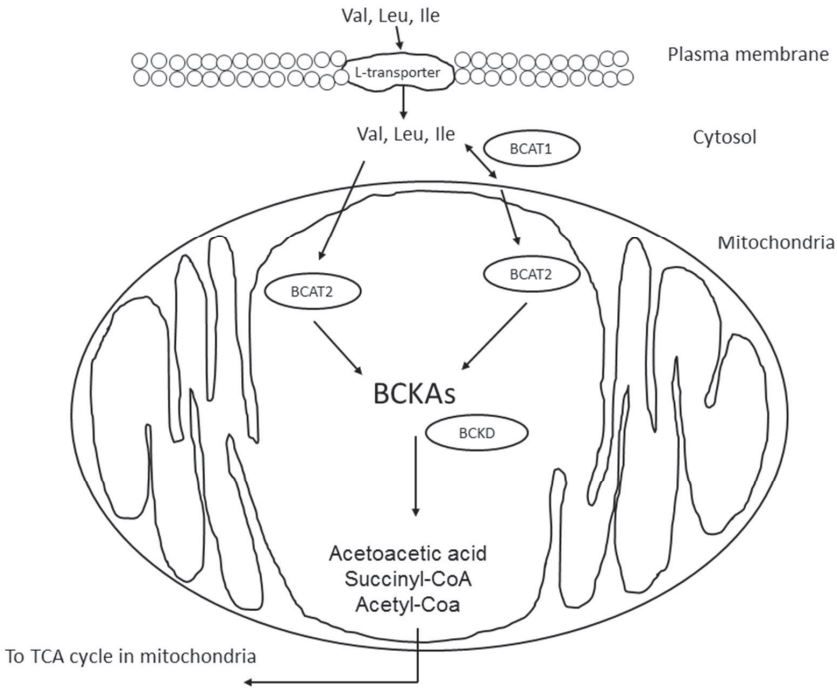


Figure 5: *Branched chain amino acids (BCAAs) valine (Val), isoleucine (Ile) and leucine (Leu) enter the cell through L-transporter in the plasma membrane. In the cytosol the BCAA aminotransferase BCAT1 and BCAT2 in the mitochondria initiate the BCAA catabolism by converting the BCAAs to branched-chain α keto-acids (BCKAs). The branched-chain alpha-keto acid dehydrogenase complex (BCKD), with additional enzymes, catalyzes the conversion of the BCKAs to Acetoacetic-acid, Succinyl-CoA and Acetyl-Coa, which can be further metabolized in the cell or can enter the TCA cycle in mitochondria for energy generation.*

2.4.5. Glucose metabolism

In the fed state, insulin binds to its receptors on the adipocyte surface and signals the translocation of GLUT4 glucose-transporters to the membrane (174). This allows for the glucose to enter the adipocyte. In glycolysis, glucose is metabolized into Acetyl-CoA and ATP in the cell cytosol. Glucose is also converted into glycerol-3-phosphate, which serves as the basis of adipocyte triglyceride synthesis. Circa 20-25% of the glucose taken up by adipocytes is used for triglyceride synthesis and storage (175). Adipocytes lack the enzyme glycerol kinase. Thus, they cannot convert the glycerol that is formed in lipolysis into glycerol-3-phosphate (176). Therefore, glucose uptake into adipocytes and its modification into glycerol-3-phosphate is essential for adipocyte lipid packaging. When glucose levels are low however, adipocytes can use non-carbohydrate substrates

like lactate or amino acids to generate glycerol-3-phosphate by glyceroneogenesis (177). If insulin receptor is knocked out in mice, glucose uptake and triglyceride synthesis in adipose tissue are reduced and the mice become resistant to diet-induced obesity (178).

2.4.6. Adipokine secretion

Adipose tissue has only lately been shown to secrete a huge variety of adipocytokines that play a role in energy intake and energy expenditure, body weight, glucose and lipid metabolism, insulin sensitivity and inflammation (179, 180). These adipokines also function in the formation of new preadipocytes (181) and regulate the migration of the cells in adipose tissue (182). Many of these adipocytokines act as hormones (endocrine action), or in autocrine or paracrine fashion within the adipose tissue and its cells.

Adipocytokines are defined either as factors secreted by adipocytes or factors secreted by any cells present in adipose tissue. The latter definition includes also the SVF cells (183, 184). Main adipocytokines include the energy intake regulator leptin, energy regulator and anti-inflammatory factor adiponectin, acute phase proteins like plasminogen activator inhibitor-1 (PAI-1), blood vessel vasoconstrictor angiotensinogen, transforming growth factor-β (TGF-β), prostaglandins, acylation stimulating protein (ASP-1), adipophilin, and factors affecting adipose tissue proliferation, heterogeneity and distribution like insulin-like growth factor I (IGF-I), glucocorticoids and sex steroids (185). Adipose tissue also secretes regulators of lipoprotein metabolism; apolipoprotein E (apoE), lipoprotein lipase (LPL) and cholesteryl ester transfer protein (CETP) (185). Many cytokines, chemokines and inflammatory-related factors are secreted by adipocytes. These include resistin, osteopontin, TNF-α, IL-6, IL-8, IL-10, IL-1α, monocyte chemoattractant protein-1 (MCP-1), interferon-γ-inducible protein 10 (IP-10), macrophage inflammatory protein-1β, granulocyte colony stimulating factor (G-CSF) and alkaline phosphatase (ALP) (133, 185, 186).

Leptin and adiponectin are exclusively secreted from adipocytes. Leptin is a 167 amino-acid long protein and hormone that regulates energy intake, energy expenditure and whole-body energy balance, but also cytokine secretion, phagocytosis, angiogenesis and reproduction (53). Leptin works as a general signal on energy reserves (187). Leptin increases food intake and decreases energy consumption by acting both on hypothalamic cells and target tissues. Thus it indirectly increases insulin sensitivity (188, 189). Leptin stimulates lipolysis by increasing cyclic adenosine monophosphate cAMP concentrations in the cell (190). Leptin can also activate inflammatory cytokine secretion (191) and inflammatory cytokines TNF-α and IL-6 in adipose tissue stimulate the production of leptin (192).

Adiponectin is a 244-amino-acid long protein that has four regions; a globular part, a region with similarity to collagen proteins, a signal sequence region important in the secretion of adiponectin from the adipocyte and a region which varies between species. Adiponectin signals through ADIPOR1 and ADIPOR2 receptors (193). Adiponectin stimulates fatty-acid oxidation, improves glucose metabolism and insulin sensitivity in adipose tissue, and decreases plasma FFA levels (194) by inhibiting lipolysis in adipose tissue (195). Adiponectin also enhances whole-body insulin sensitivity by reducing hepatic glucose production and increasing fatty-acid oxidation in adipose tissue, liver and muscle (194). Adiponectin works as an anti-inflammatory molecule. In mouse

preadipocytes, inhibition of both lipolysis and inflammatory response were observed after administration of adiponectin (196). In mice with induced adiponectin deficiency, plasma and adipose tissue TNF-α levels rise, decreasing with adiponectin treatment (197). A link between adiponectin and mitochondrial function was shown by Koh et al 2007 who demonstrated that a decrease or an increase in mitochondrial biogenesis directly inhibited or enhanced adiponectin secretion and synthesis in adipocytes (198). In addition, animal studies have indicated that adiponectin increases mitochondrial biogenesis and oxidative capacity in skeletal muscle (199, 200). This happens through triggering Ca2+ influx in the cell by ADIPOR1 receptor and resulting in enhanced AMPK and PGC-1α activity and increased mitochondrial biogenesis (200). Adiponectin also promotes cellular survival and mitochondrial proliferation by stimulating ceramidase activity through ADIPOR1 and ADIPOR2 receptors (201). This leads to reduced levels of ceramide (202), which is metabolically harmful for the cells, as well as to the formation of anti-apoptotic metabolites sphingosine and sphingosine-1-phosphate (201).

2.5. The role of adipose tissue and its mitochondria in the pathogenesis of obesity-related metabolic disturbances

2.5.1. Adipocyte hypertrophy and hyperplasia in obesity

Adipose tissue enlarges by hypertrophy, hyperplasia or both. Increased adipocyte size (hypertrophy) is associated with insulin resistance (203-205) dyslipidaemia and hepatic steatosis (206), and predicts the onset of type 2 diabetes (5, 207). Large adipocyte size has been linked to cell death (208, 209) and suggested to be the determining factor of inflammatory macrophage infiltration in adipose tissue (208).

Hyperplasia in turn is thought to be a mechanism that could protect (210) from the metabolic complications of obesity. Hyperplastic changes seem to retain insulin sensitivity (204, 211) and a favorable secretion of signaling molecules in adipose tissue (212). A reduced total number of adipocytes (less hyperplasia) in adipose tissue however is associated with type 2 diabetes (213). It has been thought that failure of adipocyte proliferation, differentiation and new preadipocyte recruitment results in excess adipocyte hypertrophy and this in turn to insulin-resistant adipocytes (210). Hyperplasia has been associated with preferential accumulation of subcutaneous adipose tissue and a “pear” shaped more healthy fat phenotype (214-216), while adipocyte hypertrophy is commonly connected with visceral fat accumulation, the “apple” shaped body image and metabolic complications (215, 216). Recent evidence suggests that rather than the anatomical position, the different characteristics and expandability of visceral and subcutaneous adipose tissue determine their effect on metabolism (217, 218). However, even though subcutaneous fat has a better adipogenic potential than visceral fat (219-221), both adipose tissue depots are capable of hypertrophy and hyperplasia during weight gain (221, 222). While there are sex- (223) and depot-specific differences in body fat accumulation (220, 221), the acquired or genetic nature of the individual differences in the hypertrophic and hyperplastic capacity of adipose tissue have still remained unclear.

Figure 6: Adipocyte hypertrophy and hyperplasia

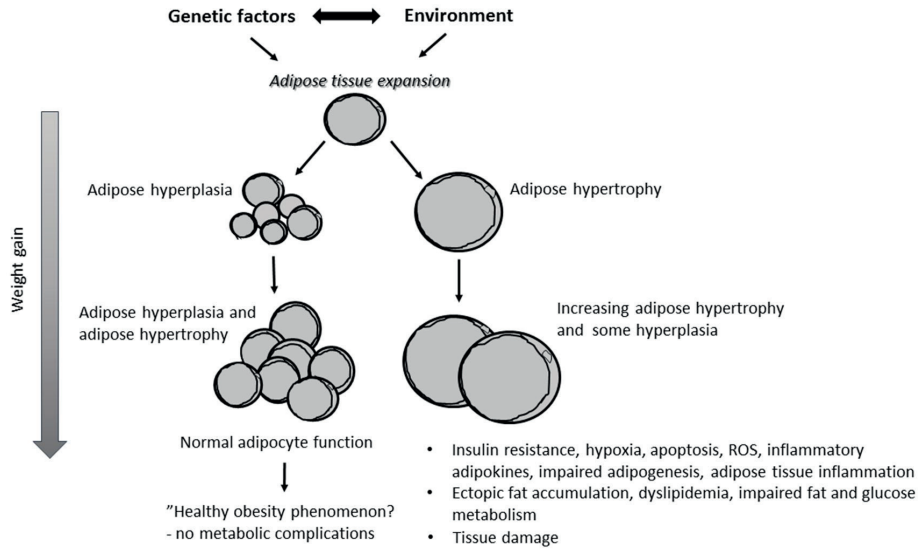


Figure 6: *Adipose tissue enlarges by increase in the size of the existing cells (hypertrophy), by recruitment of new adipocytes (hyperplasia), or by both. Genetic and environmental factors affect these capacities. With increasing weight gain adipose hyperplasia with adipose hypertrophy seem to retain the metabolic function of the cells. Adipose hypertrophy with only little hyperplasia in the tissue in turn is related to worsened metabolic parameters, insulin resistance, inflammation, impaired adipokine secretion and possibly to the limited expansion capacity of the adipose tissue and the accumulation of ectopic fat. The genetic and environmental determinants and the interindividual differences in the hypertrophic/hyperplastic capacities have remained unknown.*

Arner et al demonstrated that after bariatric surgery, subjects with the largest reduction in adipocyte size gained most in insulin sensitivity (205). Indeed, smaller adipocytes have been thought to be more insulin-sensitive (49) and are reported in the visceral fat of insulin sensitive obese individuals (14) and individuals characterized as "metabolically healthy" obese (224). Insulin and its increased concentrations in the circulation stimulate adipocyte hypertrophy (225), but not hyperplasia, because preadipocytes exhibit only low levels of insulin receptor (226). Interestingly, two studies have presented that insulin resistance (227), and also adipokine secretion (186), may depend on the cell-size distribution of large and small cells in adipose tissue, rather than only the mean cell size.

In addition to adipose tissue insulin resistance, hypertrophied adipocytes (by mean and maximal cell diameters or volume) have been associated with elevated levels of circulating inflammatory markers, and an increased number of macrophages in adipose tissue (186, 228). Studies with mice models of obesity have linked adipocyte hypertrophy with cell death (208, 209, 229). Morphologically dying

or dead adipocytes are surrounded by infiltrated inflammatory macrophages and so called crown-like structures (CLS), found in obese adipose tissue and increasing over time (208). In rodents, large adipocytes secrete more inflammatory TNF-α (230), and the expression and secretion of proinflammatory adipokines such as IL-6, TNF-α, IL-8, MCP-1, CRP, chemerin, progranulin and granulocyte colony stimulating factor have been related to adipocyte hypertrophy (186, 231, 232) and to the insulin resistant obesity state (14). Large adipocytes also release more FFA that can activate the TNF-α inflammatory secretion from macrophages (233). Adipocyte apoptosis has also been suggested as a key event that contributes to macrophage infiltration into adipose tissue and subsequent insulin resistance (234).

Mitochondria are essential in adipocyte differentiation and adipogenesis (6). Thus, they do have an important role in the hyperplastic capacity of adipose tissue. How adipocyte size relates to mitochondrial biogenesis and function has thus far been unknown. There is still very little evidence if smaller adipocytes have more active mitochondria, or contain more mitochondria than larger cells. Two recent studies did not find differences in the respiration capacities of large and small adipocytes (10, 235), but the relationship of cell size, adipocyte dysfunction and mitochondrial biogenesis still needs further studies.

2.5.2. Adipose tissue dysfunction in obesity

Adipose tissue dysfunction has been recognized as an important contributor to the obesity-related disorders (7, 8). Excess adipose tissue is associated with marked changes in the adipose tissue function and secretion, with the development of low-grade inflammation and an increased risk for developing insulin resistance, type 2 diabetes, dyslipidemia, hypertension, metabolic syndrome, coronary heart disease, stroke and vascular complications (236, 237). Pro-atherogenic, pro-diabetogenic and pro-inflammatory cytokines are secreted, accompanied by a decreased production of protective adiponectin (237).

The ability of adipocytes to expand their lipid content, while maintaining insulin sensitivity, has a protective and adaptive role in whole body metabolism (238). If the storage capacity is exceeded, lipids spill over to ectopic sites in liver, muscle and pancreas causing dysfunction and insulin resistance (47). A general assumption is that at a certain adipocyte hypertrophy level the cells begin exhibiting signs of stress. The ultimate expansion limit of subcutaneous adipose tissue however remains unknown, if any. Also, the underlying causes for the adipocyte stress have until now remained unanswered. Mechanical stress for the adipocyte membrane, mitochondrial dysfunction (6), endoplasmic reticulum (ER) stress (239), increased hypoxia of the cells (240, 241), insulin resistance, increased production of reactive oxygen species (ROS) by mitochondria (242), altered adipokine signalling, increased inflammation in the tissue (228) as well as increased possibility for apoptosis (234) have all been suggested as contributing factors for adipose tissue dysfunction. The relationship and timetable of these changes is yet largely unknown. What is known however, is that if adipose tissue mitochondria are stressed, they release more ROS, which can damage the mitochondria and other cellular entities, as well as reduced mitochondrial function may hinder many of the basic functions of an adipocyte (242). If the mitochondria are not able to cope with increased demand for FFA oxidation in obesity (65), incomplete β-oxidation products (243), and increased

endoplasmic reticulum stress in mitochondria and adipocytes may lead to a deterioration of insulin sensitivity and to other complications (244). The ultimate causality between all the contributing factors has remained unanswered.

Figure 7: Adipose tissue dysfunction and metabolic health

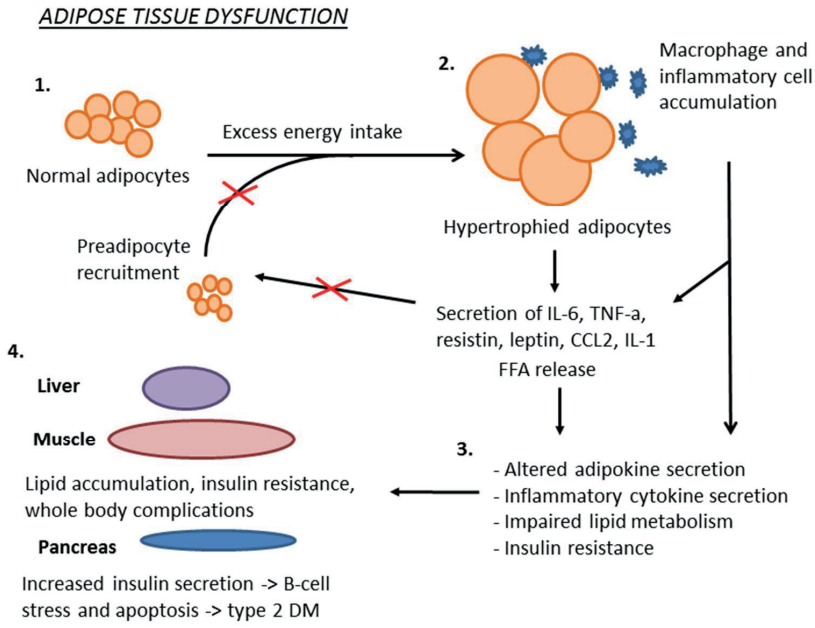


Figure 7: *Long term excess energy intake causes weight gain and adipocyte hypertrophy. Adipose hypertrophy is related to impaired adipocyte function, with impaired lipid handling and impaired secretion of adipokines, with a shift towards a pro-inflammatory state. The secreted adipokines further boost the infiltration of resident and circulating macrophages and inflammatory cells into adipose tissue. The inflammatory cytokines produced further impair preadipocyte recruitment and differentiation. Increased inflammatory secretion and impairments in the lipid metabolism cause insulin resistance in adipose tissue. Increased FFA (free fatty acid) spillover to other tissues, adipose tissue insulin resistance and dysfunction, and altered inflammatory and adipokine secretion from adipose tissue is thought to lead to FFA accumulation in ectopic sites and to whole-body metabolic complications. In muscle and liver increased lipid content and insulin resistance is observed, together with insulin resistant adipose tissue contributing to the rise in blood glucose. In pancreas, increased glucose concentrations cause β-cell overfunction and subsequently, exhaustion.*

2.5.3. Mitochondria in obese adipose tissue

Several animal studies using genetic or dietary models of obesity have shown a reduction in mitochondrial oxidative capacity in white adipose tissue (66, 104). Mitochondrial number is shown to be reduced by 50%, and the transcription of genes encoding mitochondrial proteins decreased in white epididymal adipocytes obtained from ob/ob mice when compared with lean mice (142). When these cells were treated with rosiglitazone, a PPARγ agonist and insulin sensitizer, they displayed markedly enhanced oxygen consumption and increased palmitate oxidation as well as increase in the expression of half of the previously downregulated mitochondria-related transcripts (142). In another study on mice, impaired mitochondrial function induced endoplasmic reticulum stress. Treatment with rosiglitazone resulted in enhanced mtDNA content, expression of mitochondrial proteins, fatty acid oxidation, and oxygen consumption (198). In both db/db diabetic mice and obese high-fat fed mice the mtDNA amount and the expression of genes involved in mitochondrial ATP production and genes encoding mitochondrial ribosomal proteins and mitochondrial heat-shock proteins were downregulated compared with non-diabetic or lean mice, respectively (67). Also an increase in mitochondrial mass, modifications in mitochondrial structure and enhanced expression of mitochondrial inductor PGC-1α were observed after treatment with rosiglitazone. In mice fed with high fat diet, a decrease in citrate synthase activity, reduced expression of PGC-1α and a decrease in the abundance of several proteins in the mitochondrial respiratory chain or matrix, without a change in mitochondrial mtDNA amount or basal oxygen consumption has been reported, suggesting downregulation of mitochondrial activity at the nuclear level (245).

The improvements in mitochondrial respiration after insulin sensitizer in mice studies speak for a relationship between mitochondrial function and insulin sensitivity. A study in 3T3-L1 mouse preadipocytes linked mitochondrial function to insulin signaling in adipose tissue by demonstrating that reduction in mtDNA levels and respiratory chain activity enhanced insulin signaling, but caused impaired insulin responsiveness by decreasing the glucose transport (GLUT4 translocation on the cell surface) in adipose tissue (246). Recently, another study however showed that respiratory capacity measured by cell respirometer in subcutaneous and visceral fat would be reduced in murine obesity, independent of insulin resistance (247).

Only few studies have investigated the effects of obesity or type 2 diabetes on the oxidative capacity of mitochondria or vice versa in human adipose tissue. Diverse mitochondrial parameters like structure, function and number vary between insulin resistant and insulin sensitive individuals, suggesting that low mitochondrial function is related and may contribute to insulin resistance (248). Obesity is thought to lead to mitochondrial dysfunction and impaired glucose and lipid metabolism in adipose tissue in humans (6, 64, 65). The expression of the main mitochondrial regulator PGC-1α has been shown to be decreased in morbidly obese versus lean subjects (202). Chattopadhyay et al 2011 showed that the activities of the respiratory complexes I-IV, mitochondrial membrane potential and inorganic phosphate utilization were reduced in subcutaneous adipose tissue in obese patients, and that the reduction was similar in obese versus obese diabetic patients (249). These results suggest that obesity per se relates to impaired mitochondrial function. Reduction of respiration was more pronounced in visceral than in subcutaneous fat. Flachs et al found that n-3-polyunsaturated fatty acids, known to prevent obesity and insulin resistance, upregulated mitochondrial biogenesis in adipose tissue in humans (250). Reduced mtDNA levels have been

observed in identical obese twins compared with their leaner counterparts (4), emphasizing that environmental acquired effects contribute to the regulation of mitochondrial mass and biogenesis. MtDNA copy-number correlates with lipogenesis in adipose tissue and the mitochondria have an active role in the cell enlargement and the adipocyte energy deposition process (251, 252).

The mechanisms responsible for the reduced mitochondrial oxidative capacity in adipose tissue in obesity are not yet known. It has been suggested that they involve reduced mitochondrial biogenesis and impaired mitochondrial dynamics, increased inflammation, damage caused by excess ROS in mitochondrial metabolism and ER stress, among others (253). The causality of the changes in mitochondria and the relationship to the development of the metabolic complications of obesity has also remained unclear. In adipose tissue, high-fat-fed mice displayed mitochondrial dysfunction that was observed as a secondary feature after the development of insulin resistance (105, 254). In rodent studies TNF-α has impaired mitochondrial biogenesis and function in adipose tissue (253). Also in humans, treatment of preadipocytes with the inflammatory cytokine TNF-α reduced the expression of OXPHOS genes in healthy individuals, suggesting a role for inflammation in the development of mitochondrial dysfunction (255). However, other studies speak for the mitochondria as the underlying mechanism behind insulin resistance and inflammation. High levels of FFAs and glucose have been reported to directly stimulate mitochondrial dysfunction in 3T3-L1 adipocytes (256). TNF-α and other inflammatory cytokines can also be secreted because of the dysfunctional mitochondria. As mitochondria are the site of phospholipid synthesis of the adipocyte cell membrane (153), and that adipocyte cell membrane lipid modifications have been shown to recruit inflammatory cells to adipose tissue (19), mitochondria and their dysfunction may be suggested to lie behind the changes in the cell membrane and the inflammatory processes of the adipose tissue. The causality of the various changes in mitochondria in obesity has remained to be determined. Also it is not yet known how the "dysfunction" in mitochondria manifests; is there a reduced number of mitochondria in obesity, downregulation of mitochondrial oxidative metabolism, downregulation of mitochondrial biogenesis, other functions, or all, and which of these contribute to the dysfunction of the adipocyte cell?

2.5.4. Mitochondria in obese adipocytes

Adipose tissue includes both adipocytes and stromal vascular fraction cells and thus many of the changes seen in obese adipose tissue may be derived from other cells than adipocytes. It is not yet known, how much adipocytes contribute to the mitochondrial dysfunction and metabolic disturbances in obese adipose tissue and which part of the observed mitochondria-related changes derive from adipocyte mitochondria. Although there is no consensus yet, it generally appears that mitochondrial amount and/or function are decreased in adipocyte cells.

The studies on adipocyte mitochondria are still sparse. Reduction of mitochondrial biogenesis by deletion of mitochondrial transcription factor A altered the expression of electron transport chain proteins, decreased complex I activity and enhanced reactive oxygen production in mouse adipocytes (257); however, endogenous uncoupling, β-oxidation and basal and insulin-stimulated glycolysis were increased. In humans, preadipocytes differentiated *in vitro* from human subcutaneous adipose tissue of obese persons had lower oxygen consumption rates after beta-

adrenergic stimulation compared with preadipocytes from lean subjects (68). Another recent study in humans has shown that both mitochondrial oxygen consumption rates and the levels of OXPHOS complexes CI and CIV were reduced in adipocytes in obesity, independent of the adipocyte cell size (235). Both Fischer et al and previously Yin et al have reported reduced mitochondrial oxygen consumption in obese adipocytes (10, 235) and proposed that the perceived reduced oxidative capacity of mitochondria in obesity is not due to adipocyte cell size, but rather related to overall adiposity. The above-mentioned studies are performed in obese and lean unrelated individuals. Even though previous studies have indicated a reduction in the number of mitochondria and/or mitochondrial respiratory capacity, controversy remains if there is reduction in both or only one of these factors in obese adipose tissue. Although adipose hypertrophy associates with an unhealthy metabolic obese profile (231) it is not yet known if mitochondrial biogenesis and oxidative pathways associate with adipocyte hypertrophy or to worsening of the metabolic profile in obesity in general and whether there are interindividual differences in the mitochondria-related metabolic responses to obesity.

2.5.5. Mitochondrial oxidative metabolism in obesity

Mitochondrial fatty acid oxidation has been suggested to be compromised in obesity (65). Obese individuals seem to have decreased fatty acid oxidation at least in their subcutaneous adipose tissue; however in visceral adipose tissue a study by Auguet et al found no differences between the lean and the obese groups (258). Increased fatty-acid oxidation and increased lipolysis in adipocytes have been shown to improve insulin sensitivity and reduce inflammatory signaling (259). Targeting PPARγ, a master regulator of mitochondrial biogenesis and adipogenesis by thiazolidinediones, has proven effective in the treatment of diabetes (142, 260), although some of the effect may also come from PPARγ independent direct actions of thiazolidinediones on mitochondria (261). The exact role of fatty acid oxidation in relation to whole-body metabolism in obesity, however, remains to be determined.

Adipose mitochondria are important in the BCAA oxidation and studies have demonstrated downregulation in the expression of adipose tissue BCAA metabolizing enzymes in insulin-resistant state and obesity (166, 262). In turn, after gastric bypass and related weight loss, the adipose tissue BCAA oxidation enzymes increase and circulating BCAA levels decline. BCAAs in circulation correlate positively with insulin resistance (263) and type 2 diabetes (264). Increased levels of BCAAs have also been linked to cardiovascular disease and metabolic syndrome (265, 266). However, some controversy exists, because BCAA-rich diets in mice have been associated with positive effects on glucose homeostasis and body weight (267). Furthermore, a knock-out of mitochondrial BCAA transferase in mice was associated with elevated levels of BCAAs in circulation but with improved insulin sensitivity and increased energy expenditure (268). One hypothesis for the role of BCAAs in the development of insulin resistance is that the increase in BCAAs in the cell could promote the accumulation of mitotoxic metabolites and thus pancreatic β-cell dysfunction, stress signaling and apoptosis (167). In contrast however, insulin resistance is shown to cause impairment in protein degradation of the tissues (167) and may as a result increase the levels of amino acids and BCAAs in the circulation. The causal role of BCAAs in the development of type 2 diabetes and metabolic diseases has thus remained unanswered. It has been

proposed that the BCAAs could as markers reflect the insulin resistance and the obese state of the tissues, resulting from an altered appearance and clearance of these metabolites (167).

2.5.6. AT oxygen deprivation and mitochondrial respiration

Excess adipocyte hypertrophy has been suggested to cause adipose tissue oxygen deprivation, because the adipocytes enlarge beyond the oxygen diffusion limit of circa 100μm (269). Adipose tissue hypoxia and oxygen deprivation of the cells may in turn be a factor contributing to the adipose tissue dysfunction and the inflammatory response of the adipose tissue in obesity (241, 270). The view of hypoxia as one of the inducers of inflammation in obese adipose tissue has been backed up by studies where human obese adipose tissue SVF cells cultured in hypoxic conditions secreted more inflammatory markers and harbored an increased inflammatory cell infiltrate (271). However, the role of mitochondria in this low oxygen supply milieu is not yet clear.

Partial oxygen pressure (pO2) is the difference between O2 delivery and O2 consumption in the tissue. Human abdominal subcutaneous adipose tissue pO2 lies between ~3 and 11% O2 (~23–84 mmHg) (213, 272) and blood flow in the tissue is an important determinant of pO2. Adipose tissue blood flow is shown to be lower in obese compared with lean individuals (272, 273). In rodent models of obesity, rapid weight gain was accompanied with increased expression of genes related to hypoxia, lower partial oxygen pressure and increase in hypoxic areas in adipose tissue (274, 275). In human obesity, studies are still rare and controversial. Pasarica et al reported that oxygen pressure in adipose tissue was reduced in obese versus lean individuals (213), however a study by Goossens et al found an increased oxygen pressure in obese adipose tissue (272). The latter study was matched for age, gender, presence of type II diabetes and ethnicity. Recently, in a study by Hodson et al no significant differences were found between obese and lean persons in relation to secretion of adipose tissue hypoxic markers lactate and pyruvate (276). Based on the controversial results it has been suggested that the metabolic rate of the adipose tissue may be reduced in obesity, rendering higher adipose tissue partial oxygen pressure despite the lower blood supply (9). In mouse models of obesity and type 2 DM, mitochondrial biogenesis, mass and oxygen consumption were reduced in adipose tissue of obese mice and enhanced by rosiglitazone (67, 142), supporting the idea of lower oxygen consumption in obese adipose tissue. Also in humans, metabolic pathways related to mitochondria have been shown to be downregulated and inflammatory pathways upregulated in subcutaneous fat (4), and in both subcutaneous and visceral fat (277) of obese individuals. These studies speak for the role of low mitochondrial oxidative metabolism behind the low oxygen consumption and high partial O2 in adipose tissue. However, the mechanisms behind the low mitochondrial respiration in obesity are still to be studied.

2.5.7. Adipose tissue angiogenesis

Enlarging adipose tissue needs new blood vessels. The blood vessel formation, angiogenesis, is crucial for tissue growth, repair and expansion (278). The changes in the production of angiogenic factors and inhibitors like vascular endothelial growth factor A (VEGFA), adiponectin, fibroblast growth factor (FGF) and thrombospondin 1 (279) result in an angiogenic phenotype in the tissue. In

growing adipose tissue angiogenesis contributes to adipogenesis by supplying more nutrients, growth factors and oxygen in the blood (280). Endothelial cells in new blood vessels have been shown to have stem-cell-like properties that allow them to differentiate into new preadipocytes (123). New vessels also enable the infiltration of macrophages and monocytes into the tissue, the amount of which is observed as increased in obese individual's adipose tissue (281). The changes in the vasculature may alter blood perfusion in adipose tissue, which may control the number and sizes of adipocytes, thus leading to expansion or shrinkage of certain adipose depots.

While angiogenesis is required for normal maintenance and enlargement of the tissue, in obesity it may also be pathological. In obesity, alterations of the vascular endothelium in adipose tissues include impaired vasodilation, a possible hypoxia-induced angiogenesis, and inflammation-induced vascular damage (282). The possible hypoxia in adipose tissue of obese individuals has been suggested to have a causal role in switching on inflammation and angiogenesis in the tissue. Hypoxia induces hypoxia-inducible transcription factor 1 (HIF1) and HIF2, which both increase the expression of angiogenesis-related factors and downregulate the inhibitors of angiogenesis (279). However, the connection of impaired angiogenesis and hypoxia in obese adipose tissue is still debated, because based on most recent controlled studies on adipose tissue oxygen tension (272, 276), there is no hypoxia in obese adipose tissue.

Impaired vascular function has been suggested to lead to alterations of lipid metabolism and the development of insulin resistance in tissues and in whole body, and contribute to the development of type 2 diabetes (283). While angiogenesis is also needed for energy expenditure (280, 284), recent discussion has focused on the issue, if increasing or decreasing adipose tissue angiogenesis might have beneficial effects on treating obesity.

2.5.8. Cell death

Controlled cell death, apoptosis, is both beneficial and necessary for the tissue, playing an important role in tissue homeostasis and development. Cell death can also be disrupted and harmful, at least if uncontrolled. In apoptosis, there are two principal pathways, the intrinsic or mitochondria-mediated pathway and the extrinsic, death-receptor-mediated pathway (96). Mitochondria are essential in activating the cell death apoptotic pathway and their own mitophagy (mitochondrial apoptosis) upon increased cellular and mitochondrial stress (285).

Some studies have supported the model in which adipocyte apoptosis in obesity would result from inability of adipose tissue enlargement and precede the adipose tissue macrophage infiltration and along with it, insulin resistance, dyslipidemia and other metabolic abnormalities (234). Autophagy, the controlled cell death, has been shown to be increased in adipose tissue samples from human obese and type 2 diabetic subjects, particularly in those individuals that had a greater degree of insulin resistance (286, 287). Adipocyte hypertrophy and the degree of obesity correlated with the increased expression of autophagy-related regulatory genes (286). In murine obesity, frequency of adipocyte cell death increases with increasing obesity (209). This cell death has been thought to result in the formation of "crown-like structures", characterized by dead adipocytes surrounded by macrophages – a feature that has been described in adipose tissue from both obese mice and humans

(208, 209). On the other hand, in a study by Fischer-Posovszky et al, targeted deletion and apoptosis of adipocytes in mice was sufficient to lead to a large increase in infiltration of macrophages to adipose tissue. However, these cells had an anti-inflammatory phenotype contrary to the inflammatory phenotype that is usually activated in obesity. The authors thus suggest that at least controlled apoptosis is a healthy process in adipose tissue, and other mechanisms are needed to activate the inflammatory phenotype of obese adipose tissue (288).

In obesity, multiple factors including insulin resistance, inflammation and oxidative stress are simultaneously present in adipose tissue and it is not known if the increased autophagy or cell death are a general response to the adipocyte stress and inability of adipose tissue to enlarge in obesity, or dependent primarily on some of these or other factors. In obesity, mitochondrial stress may lead to increased ROS, which further impair mitochondrial and cell function (242), predisposing to cell death and consequently to increased immune cell infiltration (208).

2.5.9. Altered cytokine secretion and low-grade inflammation in obese adipose tissue

Several pieces of evidence support the view that obesity and metabolic syndrome are chronic low-grade inflammation states (289). Obesity is often characterized by a slight increase in circulating inflammatory markers but with no visible clinical signs of inflammation (290). Modestly elevated levels of circulating inflammation markers including TNF-α, IL-6, IL-8, with disturbed secretion of plasminogen activator inhibitor-1 (PAI-1), angiotensin-II, leptin, and decreased levels of protective adiponectin (270, 291) have been recorded in obese adults.

Already in the 1970's infectious states such as sepsis were noticed to induce whole-body insulin resistance (292). In obesity, the chronic low-grade inflammation is thought to play a central role in the development of whole-body insulin resistance (36) and other complications of obesity (293). The increased infiltration of immune cells into adipose tissue and the increased secretion of proinflammatory cytokines by adipocytes and inflammatory cells has been proposed as a link between excess adiposity and the development of AT and systemic insulin resistance (294).

In obesity, immune cell populations in several tissues (liver, muscle, pancreas and adipose tissue) shift towards a pro-inflammatory state with production of inflammatory cytokines (289). In adipose tissue, the dysfunction of adipocytes has been suggested to initiate the changes (289). In a normal non-obese condition adipose tissue has resident inflammatory cells that function in tissue maintenance and various other tasks (295), but with excessive fat accumulation, their amount and functions change. Obese adipose tissue is characterized by accumulation of macrophages, lymphocytes, mast cells and neutrophils (228, 296). Two types of macrophages reside in adipose tissue; type M1 inflammatory macrophages that are capable of secreting TNF-α and IL-6 and type M2 macrophages that are anti-inflammatory. In obese adipose tissue there is a switch from an M2-anti-inflammatory milieu toward an M1-proinflammatory state (289).

Obesity alters adipokine secretion in adipose tissue. The secretion of proinflammatory adipokines and leptin are upregulated and the anti-inflammatory adiponectin is downregulated (297). In normal concentrations, adiponectin represses the inflammatory pathways in adipose and other tissues, but

in obesity, this effect is blunted (237). Despite high leptin concentrations, a state of leptin resistance emerges, where the increased leptin no longer signals on excess adiposity (298). Inflammatory cytokines are secreted both from adipocytes and the resident macrophages in adipose tissue. Macrophage-derived TNF-α has been shown to activate the adipocytes in humans, induce their lipolysis and upregulate the expression of inflammatory genes like ICAM-1, IL-6 and macrophage chemo attractant protein-1 (MCP-1) (294, 299). ICAM-1 and MCP-1 are capable of inducing monocyte migration from circulation into adipose tissue and enhance their differentiation into macrophages. Obese adipocytes secrete more TNF-α in rodents (230), and IL-6, TNF-α, IL-8, MCP-1 are expressed and secreted in humans in adipocyte hypertrophy (186, 231). Large fat cells also release more FFA that can bind to macrophage receptors, activate inflammatory pathways and induce TNF-α secretion from macrophages (300). Increased TNF-α secretion from obese adipocytes first provided evidence for a functional link between obesity and inflammation (301).

The dysfunctional secretion of adipocytokines and inflammatory factors from adipose tissue is seen to play a role in the development of insulin resistance and systemic metabolic disorders (302, 303). Inflammatory cytokines secreted by adipocytes and immune cells inhibit insulin signaling (301, 304, 305). TNF-α induces lipolysis (306), decreases preadipocyte differentiation (307), induces insulin resistance by decreasing GLUT4 on the adipocyte surface (307) and decreasing the phosphorylation of the insulin receptor (305). TNF-α downregulates PPARγ, the main inducer of adipogenesis and mitochondrial function (308). Ruan et al have shown that the changes in lipogenesis and in altered inflammatory gene expression of adipocytes can be reversed when the effect of TNF-α is removed (309).

Inflammatory gene expression on the whole is increased in obese adipose tissue (4, 296). It has however been controversial if this increase derives from adipocytes, from other cells of the tissue, or both. Some studies have suggested that the increased release of interleukins and other inflammatory cytokines from adipose tissue in obesity would be because of the non-fat cells of the tissue (310). However, also adipocytes are known secretors of IL-6, IL-8, TNF-α and other inflammatory adipokines (311). Until now, the contribution of adipocytes to the inflammatory milieu of adipose tissue has remained unanswered.

The causality of the inflammatory changes in adipose tissue is only partly clear. It has been suggested that the increase in proinflammatory molecule secretion by dysfunctional adipocytes in the long term would trigger the recruitment of circulating immune cells from macrophages to T-cells and B-cells and reinforce the inflammation to systemic level (303). Membrane lipid alterations in obese adipocytes may be at least one initial factor triggering the recruitment of inflammatory cells into the adipose tissue (19). As mitochondria are an important site of membrane lipid generation (153), changes in their function may lie behind the cell membrane plasticity.

In summary, obesity induced inflammation in adipose tissue seems to be chronic, low-grade, nutrient-induced and creating a proinflammatory state in the tissue, with an influence on whole-body metabolic status.

2.5.10. Insulin resistance and the development of type II diabetes

The dysfunction of obese adipose tissue is thought to be a major factor in the development of whole-body insulin resistance (7). Insulin resistance is a state, where the response of the tissues to insulin stimulation is attenuated and where a higher dose of insulin is needed to produce the same response as in insulin sensitive state (312). Insulin resistance was first believed to be due to low affinity of insulin to its receptor or diminished insulin receptor number in the target tissues (313). It is now established that the resistance is caused by several defects in the insulin-signaling cascade of the insulin responsive tissues; liver, skeletal muscle and adipose tissue (314). Fasting serum insulin concentrations and insulin resistance tend to increase with increasing obesity (315). In clinical studies even a mild increase in BMI (2 BMI units) increased the risk of developing diabetes threefold, and by 20-fold in subjects with obesity (BMI >30) compared with those being overweight (BMI >25) (316). However, mechanisms independent of body fat also seem to be involved in the pathogenesis of type 2 diabetes, because not all obese individuals develop insulin resistance or metabolic complications of obesity (1, 2), and also lean type 2 diabetic persons can be insulin resistant (317).

In obesity-related type 2 DM the insulin resistance of the target tissues is combined with insufficient insulin secretion from the pancreatic β-cells. Excess energy is stored in subcutaneous and visceral fat depots. When adipose tissue storage capacity is exceeded, FFAs spill over to other tissues (17). With accumulating fat deposition FFAs in circulation increase causing insulin resistance of the tissues (33). Elevated amounts of triglycerides in muscle (318), liver (319, 320), and pancreas (321, 322) associate with insulin resistance. A deficit of adiponectin may contribute to the process. To counterbalance the insulin resistance and prevent hyperglycaemia, pancreatic β-cells secrete more insulin and plasma insulin levels rise. Ultimately however, for those individuals with genetic predisposition, the pancreas cannot compensate enough. This leads to β-cell exhaustion and the development of type 2 diabetes (33). Abnormal insulin sensitivity usually precedes the clinical diagnosis of diabetes, in some cases even up to 15 years (323). This reduction of insulin sensitivity in obesity is thought to progress until the pancreatic cells no longer can compensate for it (324).

Hepatic insulin resistance in itself may also contribute to the increases in fasting blood glucose. In liver steatosis the insulin resistant liver produces glucose by gluconeogenesis despite the increased circulating insulin concentrations, which should normally suppress the hepatic glucose production (317, 325). Individuals with NAFLD have been shown to have 20-35% lower suppression of hepatic glucose production in response to insulin than matched controls (326).

Adipose tissue controls the release of free fatty acids (FFAs), which in excess are suggested to be one of the major contributors of insulin resistance in other tissues (327, 328). Elevated levels of FFA for more than a few hours in circulation have been shown to cause insulin resistance, which however disappears a few hours after the decrease in FFA levels (329, 330). Thus, it has been suggested that FFAs may have an indirect role by enhancing the accumulation of other metabolites like diacylglycerol (331). Increased insulin resistance of adipose and of other tissues leads to enhanced lipolysis and FFA release from adipocytes. This is because in insulin resistant adipose tissue insulin inadequately suppresses the lipolytic adipose tissue hormone sensitive lipase (HSL), which releases FFA into circulation. Obese subjects fail to suppress the FFA release from adipose tissue after a

meal (332). Increased levels of FFA deposition reduce glucose uptake in muscle (327), stimulate glucose production in liver (330) and insulin secretion from pancreatic β-cells (333). This ectopic fat deposition has thought to lead to systemic insulin resistance. In the long term however, FFAs cause lipotoxicity and β-cell exhaustion in the pancreas (334).

In addition to FFAs, branched-chain amino acids have been suggested as mediators of insulin resistance in obesity. However, some controversy exists and recent studies have proposed that BCAAs might instead be markers of the insulin resistance of the tissues (167) (discussed in Adipose oxidative metabolism). In obesity, decreased catabolism of BCAAs in adipose tissue is observed, followed by an increase in plasma BCAA levels (167). The mechanism by which BCAAs could contribute to insulin resistance may be through the activation of the lipogenic mTORC. Increased levels of circulating BCAA leucine have been shown to activate the mTORC cascade (167), which inhibits insulin signaling in the insulin target tissues (335). mTORC positively regulates β-cell mass and function. However, a sustained activity of mTORC causes insulin resistance in the β-cell islets, reduces β-cell survival and promotes their apoptosis (336), factors contributing to the development of type 2 diabetes. The accumulation of BCAAs in obesity is suggested to be either a contributor or at least a marker of the developing insulin resistance (167).

Low-grade inflammation of adipose tissue is seen as one factor leading to adipose tissue dysfunction and consequently, whole-body insulin resistance (303). The quantity of macrophages in adipose tissue correlates with insulin resistance (294). Also, cytokines released by the inflammatory cells and by adipose tissue, such as TNF-α, IL-6 and IL-8, TGF-β, contribute to the development of adipose tissue and systemic insulin resistance (303, 337). For instance, increased levels of inflammatory cytokine TNF-α enhance triglyceride hydrolysis and inhibit the expression of genes that are essential to insulin signaling and adipocyte differentiation (309), while its knockout leads to improved insulin sensitivity in rodents (304). Hotamisligil et al have suggested that the inflammatory cytokine TNF-α may mediate the development of insulin resistance in adipose and other tissues (301). Adipose tissue secretes also anti-inflammatory factors like adiponectin, the levels of which however are decreased in obesity. Studies have linked low levels of adiponectin to the development of diabetes; both to the insulin resistance in the target tissues and to the failure of β-cell insulin secretion (338). Adiponectin seems to counteract the effects of inflammatory cytokines in pancreatic cells (338).

2.6. Fat distribution – subcutaneous, visceral, ectopic and liver fat - in the pathogenesis of metabolic complications of obesity

2.6.1. Subcutaneous and visceral adipose tissue enlargement

Subcutaneous adipose tissue (SAT) is the largest body fat reserve storing ~80-90% of the total body fat, and in total over 4-5 times larger than visceral fat mass, which stores about 10% of body fat (339). Subcutaneous fat is the main depot for removing triglycerides from circulation. Its expansion potential has been seen as protective against the metabolic complications of obesity (340). If subcutaneous fat is missing, like in lipodystrophies, excess fat accumulates to ectopic sites - internal organs and visceral depots - causing metabolic problems (47, 312). Subcutaneous fat depots have

increased rates of adipose turnover and new adipocyte formation compared to visceral fat and it is generally thought that new and 'younger' adipocytes are metabolically more healthy (3, 120, 203).

However, despite its protective nature, subcutaneous fat has also been shown to contribute to obesity-related complications. In a group of both obese nondiabetic (341) and obese diabetic (342) males, subcutaneous truncal fat played a more important role in the obesity-related insulin resistance than visceral fat. Goodpaster et al demonstrated a relationship between subcutaneous abdominal fat and insulin sensitivity (343) and the Amsterdam Growth and Health Longitudinal Study related subcutaneous fat to arterial stiffness (344).

Visceral adipose tissue (VAT) is known to be associated with metabolic syndrome and cardiometabolic risk factors (345, 346). It has higher metabolic activity measured by lipogenesis and lipolysis compared to other fat depots (48). Increased lipolysis, FFA release and inflammatory secretion from visceral adipose tissue is thought to lead to the accumulation of excess liver, muscle and pancreatic fat, inducing lipotoxicity and insulin resistance in these organs (347, 348). Men are often thought to be at more risk for metabolic complications of obesity, because they tend to accumulate visceral fat while women, despite their higher percentages of body fat compared to men, tend to accumulate subcutaneous fat (223). With accumulating age, body fat distribution often shifts from subcutaneous to visceral depots.

However, only a small portion of total body fat (7-18% depending on sex and the individual) resides in the abdominal cavity (347). Also, visceral fat contributes to only 15% of the total amount of free fatty acids in the systemic circulation, majority coming from subcutaneous abdominal or other subcutaneous depots (347, 349). This has raised questions about the actual contribution of visceral fat to total body insulin sensitivity. Recent studies present that low visceral adiposity in obese people is associated with an insulin sensitive obese phenotype (12, 13, 15, 350), often also termed as "metabolically healthy obesity". Thus, there is an idea that increased visceral fat mass might represent the inability of patients with insulin-resistant obesity to store enough fat in subcutaneous adipose tissue, leading to ectopic fat accumulation and metabolic problems (340). In contrast, a study by Fabbrini et al showed that surgical removal of omental fat did not improve insulin sensitivity in obese adults (351).

Subcutaneous and visceral adipose tissues have distinct transcriptomic characteristics. In a study by Poussin et al, a large group of genes required for the interaction between the adipocyte and its tissue environment were upregulated in both depots in high-fat fed mice, however with different individual upregulated genes (352). In visceral adipose tissue, these structural changes in tissue matrix were related to body fat gain, while in subcutaneous adipose tissue no such connection was observed. The site-specific gene expression profiles of subcutaneous and visceral adipose tissue have also been shown in humans (353). Many of the identified genes in humans that are different between subcutaneous and intra-abdominal fat are involved in developmental patterning (354), suggesting that there may be differences in the regulation of adipose tissue formation between these two depots. As preadipocytes from a certain compartment maintain their phenotypic site differences when mature, adipocytes from different depots have proposed to have different origins (355).

Subcutaneous and visceral adipose tissue produce different amounts of adipokines, hormones and inflammation markers, including leptin, angiotensinogen, TNF-α, PAI-1 (356), carboxypeptidase and thrombospondin (357). The expression of angiotensinogen for blood pressure regulation (358), complement factors (359), insulin receptor, 11β hydroxysteroid dehydrogenase (11β HSD) (360) and the acylation stimulating protein ASP are higher in visceral fat, while leptin is largely produced by subcutaneous adipose tissue (358) and TNF-α by both depots (361). In extreme obesity, IL-6 has been reported to be derived mainly from visceral adipose tissue (361). In the light of this, it seems plausible that both adipose tissue depots actively contribute to the development of, or protection, from the obesity-related metabolic complications.

2.6.2. Ectopic fat accumulation

Excess fat accumulation is thought to provoke a stress reaction in adipocytes, causing the development of inflammation and insulin resistance in adipose tissue. This results in the inability of the adipocytes to ingest more glucose to be stored as fat (7). In obesity, there is downregulation of fatty acid uptake into adipose tissue (18). When adipose tissue storing capacity reaches its limits, excess fat starts to accumulate in internal organs like liver, pancreas, muscle and visceral depots (47) causing their insulin resistance, a phenomenon called ectopic fat accumulation and lipotoxicity (312, 362). Accumulation of excess fat in ectopic non-adipose tissues leads to cell dysfunction or cell death (363). Several studies have demonstrated that lipid accumulation increases in pancreas and myocardium from lean to obese and to type 2 diabetic individuals (364, 365), suggesting that lipid accumulation precedes other metabolic problems. Interestingly, the correlation between adipose tissue mass and developing insulin resistance and other metabolic complications of obesity is not linear – even some very lean persons may develop insulin resistance, while some obese do not (366, 367). This association can even vary in populations, indicating that there may be genetic determinants behind it. (366). Apart from excess adipose tissue, also the partial or total absence of fat is associated with insulin resistance and cardiometabolic disturbances. This is a phenomenon seen in lipodystrophies, where the patients do not have enough adipose tissue to store fat (368). This notion supports the prevailing idea that when the oxidative capacity and the storage capacity of adipocytes are compromised, lipids accumulate to ectopic sites with toxic consequences (362). Adipose tissue expandability may be an important factor in determining if an individual develops metabolic complications of obesity or not (340). Obesity-associated insulin resistance and metabolic disturbances might be postponed if the adipose tissue expandability, especially in subcutaneous depots, is good (340). Until now however, it has not been known if the expanding capacity of adipose tissue (by increasing adipocyte number with hyperplasia or adipocyte size by hypertrophy) is a genetic or an acquired phenomenon. The limit of adipose tissue storage capacity may also differ between individuals. Although mitochondria have been recognized as important in the preadipocyte generation and hyperplastic capacity of adipose tissue, the genetic versus environmental reasons behind the differences in adipose tissue expandability have thus far remained unknown.

2.6.3. Liver fat accumulation

Liver plays an important role in lipid metabolism. It harvests free fatty acids (FFA) from the bloodstream, stores them as lipids and exports as lipoproteins. FFAs in the liver can be metabolized either by hepatocyte mitochondria through oxidation to produce ATP or through esterification to triglycerides, which are stored as liver fat or secreted into the bloodstream as lipoprotein particles, mainly VLDL. Fatty acids can also be used for phospholipid synthesis or ketone body production. Apart from lipids, the main metabolic functions of the liver include storing glucose as glycogen, generating it by gluconeogenesis, secreting coagulation factors and producing inflammatory cytokines (369).

In a fasting state, liver releases glucose into circulation by glycogenolysis and gluconeogenesis (370). This production is enhanced by glucagon (371) and suppressed by insulin. Fatty acids from adipose tissue are oxidized in the liver mitochondria or synthesized into VLDL (372, 373). In a caloric excess state in normal healthy subjects, increasing insulin (374) and glucose concentrations suppress hepatic glucose production (375). Liver stores dietary glucose as glycogen, oxidizes it for immediate energy needs or converts the excess amounts into fat by hepatic de novo lipogenesis (376). FFAs are used to fill the hepatic triacylglycerol stores (377).

The prevalence of fatty liver increases with obesity (378, 379). Overfeeding of 4 weeks with a high-fat high-glucose diet increased weight by 9%, but liver fat content with 2-3 fold in normal healthy subjects (380). Weight loss in turn decreases liver fat content (381). The relationship of body weight and the accumulation of liver fat however is not linear (382). Some subjects develop fatty liver at different obesity stages than others. In accordance with this, metabolically healthier insulin sensitive obese subjects have been suggested to have less liver fat than their insulin resistant peers (16, 382). A study on monozygotic twins discordant for obesity has shown that liver fat content is increased in acquired obesity independent of genetic effects (383). However, it is not known, if liver fat or some other metabolic measure distinguishes between health and disease in obesity, and if this association is genetic or acquired.

Figure 8: Liver fat accumulation

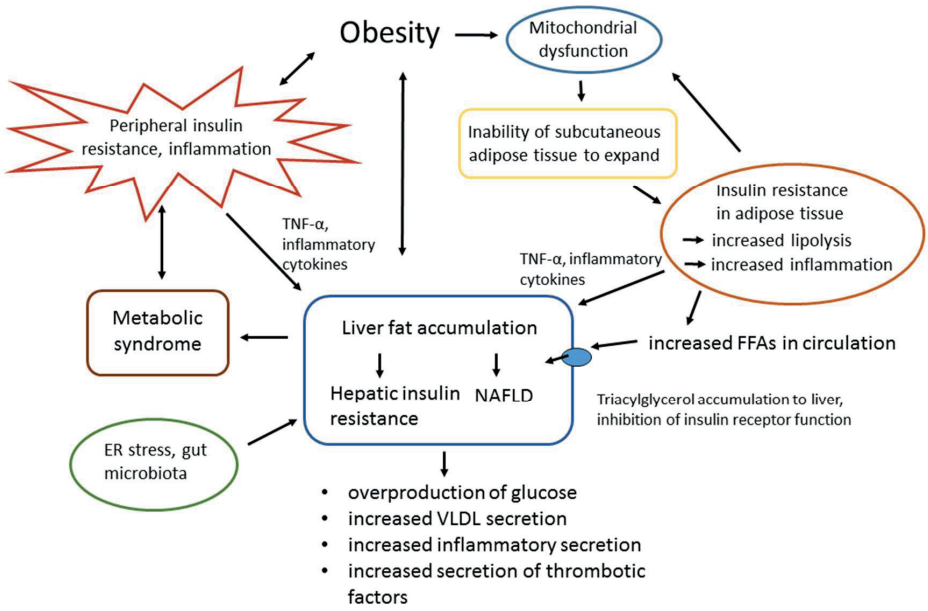


Figure 8: *Obesity and excess energy intake cause adipocyte hypertrophy and adipose tissue enlargement. Excess subcutaneous adipose tissue enlargement stresses the tissue, causing mitochondrial dysfunction, increased lipolysis, increased inflammatory secretion, altered adipokine secretion and insulin resistance of the tissue. Inability of the subcutaneous fat to enlarge sufficiently leads to increased FFA concentrations in the circulation which deposit as ectopic fat in the liver. Inflammatory cytokines secreted by adipocytes and adipose tissue inflammatory cells together with the increased fat content contribute to the insulin resistance state of the liver and the whole body. Increased liver fat accumulation, whole-body inflammation and insulin resistance present as metabolic syndrome.*

Non-alcoholic fatty liver disease (NAFLD) is defined as a state where over 5% of liver content or weight is fat. Insulin resistance and inflammatory state, caused by obesity and excess triglycerides, in adipose and other peripheral tissues such as muscle, also contribute to the increase in plasma glucose and inflammatory secretion to the circulation. These factors in turn contribute to the fatty acid deposition and development of inflammation and insulin resistance in the liver. In the insulin resistant, fatty liver, glucose production is not inhibited despite the increased blood glucose concentrations. Liver produces excess VLDL particles contributing to the hypertriglyceridemia often seen in obesity, hyperglycemia by glucose production, systemic inflammation by inflammatory molecule expression and secretion and increased probability of thrombosis by increased thrombotic factor secretion. Endoplasmic reticulum (ER) stress in adipose tissue and factors secreted by gut microbiota may contribute to the FFA deposition and insulin resistance of the liver.

In obesity, continuous accumulation of fatty acids in liver and inflammatory factors secreted by adipose tissue (384, 385) can lead to a non-alcoholic fatty liver disease (NAFLD). This comprises a spectrum of disorders from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH) and on to fibrosis and cirrhosis (386). NAFLD is defined as more than 5% fat content of liver volume or liver weight (387) and histologically when 5% or more of the hepatocytes contain intracellular visible triglycerides (388). Features of steatohepatitis include hepatocellular injury (ballooning, apoptosis and necrosis, Mallory's hyaline bodies and giant mitochondria in hepatocytes), inflammatory milieu and fibrosis (389). Many (390, 391) studies have found that VLDL-particle secretion from liver is greater in patients with NAFLD than in those with normal hepatic triglyceride concentrations. However, for some reason the increased secretion is not enough to compensate for the increased liver fat deposition.

Intra-hepatic triglyceride content has been directly linked to hepatic insulin resistance by many studies (326, 392, 393) and NAFLD associated with insulin resistance (394, 395) and obesity (392, 395). The relationship of fatty liver and insulin resistance has been suggested to be independent of total body fat, visceral fat (396) and intramyocellular fat (397). The development of insulin resistance and inflammation in the liver is thought to happen simultaneously, at the same time during the increase in liver fat content. Visceral and subcutaneous adipose tissue enlargement produces both excess FFAs and inflammatory factors, which activate cascades to impair insulin signaling in the liver (384). FFAs are released and deposited ectopically uninhibited because of the insulin resistant state in the adipose depots (398). Adipose tissue derived inflammatory factors and impaired liver function in turn has been evidenced by an increased expression of inflammatory markers in subcutaneous adipose tissue of obese women with high liver fat content compared with obese women with normal amounts of liver fat (399). Insulin resistant state in the liver and other tissues enhance pancreatic β-cell insulin secretion, and NAFLD has been associated with pancreatic β-cell exhaustion and dysfunction in obese subjects (400, 401).

In the fatty, insulin-resistant liver the insulin clearance from bloodstream is reduced (402). Inhibition of hepatic glucose production becomes impaired (326) and the secretion of VLDL lipoprotein particles is not sufficiently inhibited (391). Liver overproduces both glucose and VLDL, and plasma glucose and lipoprotein levels rise. Cytokines and coagulation factors are overproduced. The results of fatty liver - hyperglycemia, hypertriglyceridemia, low HDL cholesterol concentration and hyperinsulinemia - are commonly, but not always, seen in the obese state. Hepatic triglyceride accumulation does not necessarily cause insulin resistance, as demonstrated by mouse studies (403, 404). Thus, while those obese that accumulate ectopic fat and have hypertriglyceridemia and hyperglycemia are known to be at risk for cardiovascular disease and other metabolic complications of obesity (405), recent discussion has focused on the possibility that liver fat accumulation might distinguish between the obese developing metabolic complications from those who do not.

2.7. Metabolically healthy phenomenon in obesity

Studies in the last decades have shown that some obese humans have a better metabolic profile than what would be expected regarding their adiposity. This 'metabolically healthy -obesity' -phenotype has been described by several groups already in 1980 (406, 407). From then on, characterizing the metabolically healthy obese (MHO) phenotype and the mechanisms that would protect the obese individuals from the metabolic complications of obesity has been the subject of many studies (1, 366, 408, 409). However, there has been some disagreement regarding the characterization of MHO and thus also its prevalence, because some groups have used the term to mean absence of insulin resistance and some or all features of metabolic syndrome (1, 410). Samocha-Bonet et al have suggested that one possibility would be to look at the insulin sensitivity of the subjects only (408). In general, as presented in the meeting of IASO in 2013 on healthy obesity, the basic metabolic characteristics of the healthy obese phenotype are thought to be high level of insulin sensitivity, no hypertension, a favorable blood lipid profile, little inflammation, good hormonal and immune profiles as well as a low amount of liver fat despite the persons having excessive body fat (1). Most of the studies have reported two key characteristics of MHO. The MHO individuals present less visceral and ectopic fat, in particular in their liver, than obese persons with the same parameters of adiposity and BMI, but with metabolic abnormalities (1, 16, 408). Another key factor seems to be a lower degree of systemic inflammation (lower plasma high-sensitivity C-reactive protein) in the MHO persons compared with other unhealthier obese persons (15, 16, 411). MHO has also been associated with a lower activation of NLRP3 inflammatory cascade in macrophages of visceral adipose tissue compared with obese unhealthy individuals, as well as a generally more favorable inflammatory profile in MHO versus the unhealthy obese (412). 15–30% of the obese individuals, depending on the metabolic parameters involved and the study groups examined, do not develop alterations in their metabolic health, at least not in short term (2, 413). The reasons behind this have remained unclear. It is still not known, which factors distinguish the individuals prone to metabolic complications of obesity from those who are not.

Adipose tissue hypertrophy/hyperplasia has been postulated as one mechanism distinguishing the metabolically healthy and the unhealthy obese individuals. Metabolically healthy obese appear to have smaller adipocytes (circa 15% smaller) than the metabolically unhealthy obese (14, 224). McLaughlin et al showed that metabolically healthy obese compared with unhealthy peers have increased expression of genes related to adipocyte differentiation (227). Recently, another study found that adipose tissue extra-cellular matrix remodeling differed between the two metabolic groups and adipose tissue enlargement could thus be a factor behind the differences in metabolic health (414). The expansion of adipose tissue needs the expansion of its vasculature and maintenance of oxygen supply. A study in obese mice has shown that increasing vasculature in adipose tissue preserved adipose tissue function in obesity (415).

Differences in the visceral versus subcutaneous fat amount as well as ectopic fat accumulation have been suggested as important factors in the development of either of the phenotypes. However, these results are not without controversy. Metabolically healthy obese have been reported to have even 50% less visceral adipose tissue than the unhealthy obese (1, 11, 13). It was thus thought that the healthy obese could be able to store more fat in their subcutaneous depots (2, 11). However, not all

studies have found differences in the amount of subcutaneous fat between these metabolic groups (13, 16). However, ectopic fat accumulation in the liver and skeletal muscle has been shown to be lower in the metabolically unhealthy obese individuals compared with their unhealthy peers (1, 16). Hepatic fat content has been associated with the metabolically unhealthy obese phenotype and insulin resistance (416) and suggested as a determinant of the metabolic health (409).

Interestingly, it has been shown that the MHO phenotype may not be a lasting phase in obesity (417-419). In these three studies, it was noticed, that time (aging) and probably additional weight gain may turn the previously "healthy" obese to an unhealthy obese state. It may thus be speculated, that the "metabolically healthy" phenomenon would only be a transient phase before the measureable metabolic changes and could just depend on the stage when the individual happens to be examined.

2.8. Twin study setting in obesity research

This thesis is based on studies on young healthy obesity-discordant and obesity-concordant monozygotic twins. Monozygotic (MZ) twins are born from a single fertilized ovum and are genetically identical. Dizygotic (DZ) twins are derived from two different ova and thus share approximately 50% of their genes, as any siblings do. Usually, a twin study compares the similarity between MZ and DZ twins (420). If the MZ twins are similar in the investigated variable, but the DZ twins are less so, the difference is taken as evidence of a genetic effect. Twins often share the same environment in childhood. MZ twin pairs, who are discordant for the investigated trait, are invaluable study subjects for that trait, because they are perfectly matched for genes, age, gender and early environmental influences – and often also matched for environment in adulthood. Comparing MZ trait-discordant and MZ trait-concordant otherwise healthy twins gives a unique possibility to discern between genetic and environmental etiologies of the investigated trait. The factors behind the individual response of fat tissue to obesity, and the development of metabolic disturbances can in this setting be distinguished from genetic effects. The role of mitochondria in acquired obesity has not yet been studied in twins. The MZ co-twin control setting is ideal for discerning the factors behind mitochondrial dysfunction that are caused by obesity.

3. AIMS OF THE STUDY

The overall aim of this thesis was to study the biological pathways in adipose tissue that lead to the development of metabolic complications in obesity and to detect novel factors associated with obesity-related diseases.

Specifically the aims were

1) To study the effects of acquired obesity on adipocyte size and number and how these factors relate to the development of metabolic complications in obesity.

2) To study different fat depots and transcriptional pathways in adipose tissue related to mechanisms maintaining the "metabolically healthy obesity", where a subset of obese individuals stay free from the metabolic complications usually associated with weight gain.

3) To study mitochondrial biogenesis and oxidative metabolism in adipose tissue in acquired obesity, and the associations of these factors to adipose tissue metabolism and metabolic complications of obesity.

4) To study the effects of acquired obesity on mitochondrial biogenesis in adipocytes and compare the gene expression results and other mitochondrial parameters in adipocytes and adipose tissue.

4. SUBJECTS

4.1. FinnTwin16 and FinnTwin12 birth cohorts

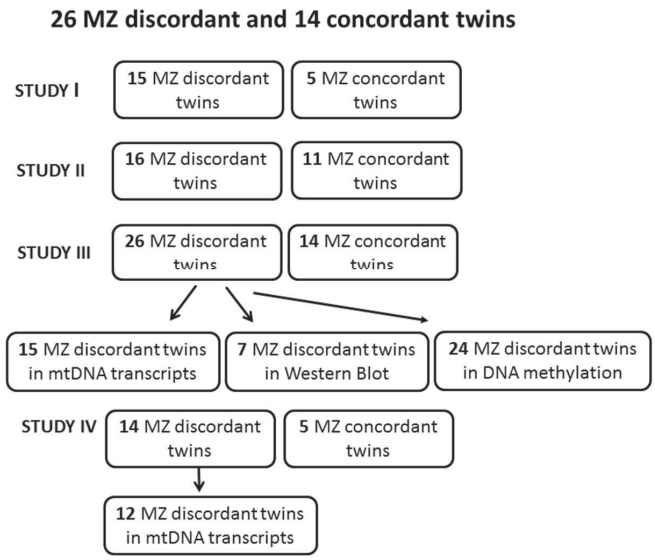
The studied twins were recruited from two population-based longitudinal birth cohorts: FinnTwin16 and FinnTwin12, all twins living and resident in the country. Originally the twins and their families were followed up and studied for health behaviors. FinnTwin16 consists of five consecutive birth cohorts (1975-1979) of Finnish twins and their families, identified from the Central Population Registry of Finland (421). Data collection was started in 1991 and the baseline questionnaire was conducted at the twins 16th birthday or in 60 days' time of it. At baseline, there were 2733 twin pairs. The respondents were surveyed again at 17 and 18.5 years and at 22-27 years (response rates 83% to 97%, n = 5,601 at baseline). FinnTwin12 also consists of five birth cohorts (1983-1987) identified from the Central Population Registry of Finland (421, 422). In FinnTwin12, of all eligible families, 87% completed the family questionnaire and thus around 2800 families were participating at the baseline. These twins were originally 11-14 years of age at the beginning of the study and were followed up at regular intervals, 11 to 12, 14, and 17.5 years of age, and a subgroup until young adulthood (20-25 years) (response rates 74% to 92%, n = 5,184 at baseline).

4.2. Subjects included in the thesis project

For the TwinFat 2008 study and this thesis project, the twins were recruited from both FinnTwin16 1975-1979 (FinnTwin16, n=2 839) and 1983-1987 (FinnTwin12, n=2 578) registries. Together, 26 rare weight-discordant (intra-pair difference (Δ) in BMI 3-10 kg/m^2, males n = 9, females n = 17, aged 29.9 ± 0.6 years) and 14 concordant (ΔBMI 0-2 kg/m^2, males n = 9, females n = 5, aged 31.6 ± 0.6 years) young healthy adult monozygotic (MZ) twin pairs between the age of 22-36 were studied. All twins were healthy with no diagnosed diseases, except that one obese co-twin had type 2 diabetes mellitus and used metformin and insulin. Another obese co-twin had an inactive ulcerative colitis and used mesalazine and azathioprine. All other subjects were healthy, normotensive and did not take any regular medications except for contraceptives. The twins' body weights had been stable for at least 3 months prior to the study. The zygosity of the twins was confirmed by genotyping ten informative genetic markers (423). Additionally, adipocytes from six obese and seven lean volunteers were used in study IV. A written consent for the study was obtained from all participants. The study protocol was designed and performed according to the principles of the Helsinki Declaration and approved by the Ethical Committee of the Helsinki University Central Hospital.

Subgroups of the twins for the four studies in this thesis were as follows (Figure 9): 15 weight-discordant and five weight-concordant MZ twins were included in study I. Study II included 16 obesity-discordant and 11 concordant twin pairs. In study III, 26 obesity-discordant and 14 concordant twin pairs were used in the transcriptomics analyses, 15 discordant MZ twin pairs in the laboratory measurements of mtDNA amount and mtDNA transcripts, seven discordant MZ twin pairs in the OXPHOS Western Blot analysis and 24 discordant MZ twin pairs for epigenetic analyses. Study IV included 14 discordant and five concordant MZ twin pairs, and additionally seven lean and six obese individual volunteers for the OXPHOS Western Blot analyses

Figure 9: Subgroups of the twins in the studies I-IV



4.3. Study design

The twins were studied extensively. Body composition of whole body fat percentage was measured by DEXA (dual energy x-ray absorptiometry), body fat distribution of subcutaneous and visceral adipose tissue were measured by MRI (magnetic resonance imaging) and liver fat by MRS (proton magnetic resonance spectroscopy). Oral glucose tolerance test (OGTT), lipids, adipokines, hs-CRP and other metabolic measurements were performed. Subcutaneous adipose tissue biopsies under the umbilicus were taken, the total RNA of adipose tissue and adipocytes extracted and genome-wide transcriptomics analyses of adipose tissue and adipocytes performed. Differences between the obese and the lean co-twins in pathways related to adipocyte morphology, metabolic disturbances, mitochondria-related nuclear and mitochondrial transcription and inflammation in subcutaneous adipose tissue and adipocytes were analyzed. Adipocytes were photographed and calculated and the diameters of the cells were measured. Total DNA from adipose tissue samples was extracted and the amount of mtDNA determined. MtDNA-encoded transcripts were measured. Western blot of total adipose tissue (twins) and adipocyte (obese and lean individuals) lysates were performed for OXPHOS complexes I, II, III, IV and V.

5. METHODS

5.1. Clinical assessments

Weight and height were measured after a 12-hour overnight fast in light clothing for the calculation of body mass index (BMI). Body composition was determined by dual-energy X-ray absorptiometry (Lunar Prodigy, Madison, WI, software version 8.8) (424), the amount of SAT and visceral adipose tissue (VAT) by MRI and liver fat by MRS with a 1.5 Tesla MRI imager (Avanto, Siemens, Erlangen Germany) (425). Parental weight, height and age were recorded at the twin age of 16.

5.2. Blood tests and analytical measurements

Concentrations of fasting plasma glucose were measured after a 12 h overnight fast, using the spectrophotometric hexokinase and glucose-6-phosphate dehydrogenase assay (Gluko-quant glucose/hexokinase, Roche Diagnostics) with a Hitachi Modular automatic analyser and fasting serum insulin with time-resolved immunofluorometric assay (Perkin Elmer). A 75 g oral glucose tolerance test (OGTT) was performed to calculate HOMA-insulin resistance index as [fasting glucose (mmol/l) x fasting insulin (mU/l)/22.5] (426), Matsuda index (427) and insulinogenic index (428). The twin with type 2 diabetes was not included in the OGTT analyses. Plasma total cholesterol, HDL cholesterol and triglyceride concentrations were determined with enzymatic methods (Roche Diagnostics Hitachi, Hitachi Ltd, Tokyo, Japan). LDL cholesterol was calculated from the Friedewald formula. Serum high-sensitivity C reactive protein (hs-CRP) was measured by particle-enhanced immunoturbidimetric assay (Cobas CRP (Latex) HS, Roche Diagnostics) on Modular automatic analyzer (Hitachi Ltd, Tokyo, Japan) and plasma leptin, adiponectin, adipsin and resistin by enzyme-linked immunosorbent assay (ELISA) by using DuoSet ELISA, R&D Systems Europe Ltd, Abindgon, UK.

5.3. Subcutaneous abdominal adipose tissue biopsies

Surgical biopsy samples of abdominal SAT under the umbilicus were obtained under local anesthesia and snap frozen in liquid nitrogen. Adipocytes were collected from fresh adipose tissue biopsies by digesting a piece of adipose tissue in 2% collagenase-DMEM/F-12. The reaction was stopped after 1h by DMEM/F-12 supplemented with 10% NCS, the solution centrifuged to produce an adipocyte upper layer and adipocytes collected and washed twice with DMEM/F-12. Based on the availability of sample material, the frozen tissue specimens of adipose tissue or the fresh adipocytes were used for transcriptomics analyses for adipose tissue (Affymetrix, all 26 discordant and 14 concordant twin pairs) and adipocytes (Affymetrix, 14 discordant and 5 concordant twin pairs), determination of the mtDNA amount in adipose tissue (15 discordant twin pairs), determination of mtDNA-encoded transcript levels in adipose tissue (15 discordant twin pairs) and adipocytes (12 discordant twin pairs) and an OXPHOS protein analysis in adipose tissue (7 discordant twin pairs). Additionally, adipocytes from six obese and seven lean individuals were used for OXPHOS analysis in article IV. 24 discordant and 11 concordant twin pairs were available for

DNA methylation analyses in adipose tissue for article III. Clinical characteristics of the selected and unselected twin pairs were similar.

5.4. Adipose tissue (AT) and adipocyte RNA extraction, transcriptomics analyses and data validation

Total RNA was extracted from the whole SAT biopsies and from the adipocytes by using the RNeasy Lipid Tissue Mini Kit (Qiagen) with a DNase I (Qiagen) step according to the manufacturer's instructions. Gene expression studies in the articles I, II and III of the thesis were done with whole AT biopsy samples that included stromal vascular fraction cells (SVFC). In article IV of the thesis, both adipocyte and adipose tissue gene expression data were used in 14 discordant twins. Total RNA (500 ng) from all twins in AT and adipocytes was used for the gene expression analysis on Affymetrix U133 Plus 2.0 array. The labeling and hybridization were done according to the manufacturer's instructions. Pre-processing of the expression data was done using BioConductor (429) and the GC-RMA algorithm in articles I, III and IV. In article II, the data were normalised using the GC-RMA algorithm and analyzed by the GeneSpring GX 7.3 software (Agilent Technologies, Santa Clara, CA, USA).

The AT data were validated with four key genes in adipose tissue in 14 discordant pairs. Individual transcript levels in adipose tissue were used for leptin (probe 207092_at), adiponectin (207175_at) and hormone-sensitive lipase (LIPE) (208186_s_at) in article II. Transcript levels of leptin, adiponectin, LIPE and MCP1 (also known as CCL2) were validated by quantitative PCR (iQ5 Real-Time PCR Detection System; Bio-Rad, Hercules, CA, USA). Samples were run in triplicate and the comparative Ct method was applied using four housekeeping genes (B2M, YWHAZ, PPIA and RPLP0). Spearman correlations between quantitative PCR and Affymetrix were: leptin $r = 0.82$, adiponectin $r = 0.71$, LIPE $r = 0.58$ and MCP1 $r = 0.94$ (all $p < 0.002$).The transcriptomics data of adipose tissue was published with article II and the data of adipocytes with article IV.

5.5. Adipocyte morphology

For the measurement and calculation of adipocyte volume and number, part of the adipose tissue sample was digested in 2% collagenase-DMEM/F-12 media for 1h and then supplemented with 10% newborn calf serum (NCS) (430). The solution was centrifuged to produce an adipocyte upper layer. After washing the adipocytes twice with DMEM/F-12 media, photographs of the adipocytes were taken with a light microscope (Zeiss, Axioplan2) using magnification 50x. Diameters of 200 cells were measured by image processing and analysis software ImageJ (ImageJ 1.42q/ Java 1. 6.0_10 32-bit) (430). Adipocyte volume and number were calculated as explained below (430) and as published before (61, 120). Mean adipocyte diameters, volumes and adipocyte number were calculated for each individual using the following formulas:

$$V = \frac{\sum_{1}^{100}\left(\frac{\pi \cdot d_i^3}{6}\right)}{100} \quad , \quad w = \frac{\sum_{1}^{100}\left(\frac{\pi \cdot d_i^3 \cdot 0.915}{6 \cdot 10^6}\right)}{100} \quad , \quad n = \frac{m(kg)}{V(dm3) * 0.915(kg/dm3)}$$

V=cell volume (μm^3), d=cell diameter (μm), w=cell weight (μg), n=total adipocyte number, where m=total body fat mass (kg) and V (m^3)*rho (density, fat) =mean weight of a single adipocyte. Density of fat cell triglycerides=0.915g/mL (22). Adipocytes were assumed to be spheres.

In the first article of the thesis, the distribution of adipocyte volumes was divided into quartiles with the lowest 25% quartile representing "small cells" (<160 pL) the mid-quartiles for the "medium" (160-330 pL) and "large cells" (330-550 pL) and the top 25% quartile for the "extra-large" cells (550-2 000 pL). The proportions of small, medium, large and extra-large cells were calculated for each individual.

The twins were also divided into two groups in regard to 'hyperplastic' and 'hypertrophic' obesity, where in the hyperplastic group the obese co-twins had responded to obesity with increasing their cell count and had more cells compared with their leaner co-twins, whereas in the hypertrophic profile the obese twins had less or a similar amount of cells than their leaner co-twins. However, all the obese co-twins had larger adipocytes than their lean co-twins. The 'hypertrophic' group in this thesis means the twin pairs where the obese co-twins had no hyperplasia and thus no increase in their cell count despite increased fat mass (relative to their lean co-twin) and exhibited relatively more hypertrophy than the 'hyperplastic' group (430).

5.6. Bioinformatics and pathway analyses

In article I of the thesis, the bioinformatics analyses were performed to find associations between obesity-induced changes in adipocyte size and number and global adipose tissue gene expression. Log 2 transformed gene expression fold changes (FC) (heavier/leaner co-twins in all pairs) were paired with log 2 fold changes (heavier/leaner co-twins) in adipocyte size. The correlation limit was set to 0.6 for both positive and negative correlation. All the resulting correlations were statistically significant with a correlation *p*-value less than 0.05. The *p*-values were then adjusted for multiple testing by pFDR (431), which results in a q-value. All the q-values were considered significant with a value of less or equal to 0.25. The computational platform Moksiskaan (432) was used to study if the identified correlating genes were involved in similar molecular circuits, how they interact with each other and which signaling pathways they were involved in. Moksiskaan constructs a network for a list of correlating genes by using literature-driven pathway information from several databases to identify known regulations between the genes in the list of interest. All the correlating genes with correlation limit +/-0.6 and p-value < 0.05 were used as an input to Moksiskaan pathway analysis. A comprehensive network was constructed by using all 206 imported pathways to identify known regulations between the correlating genes. Connections between the correlating genes were identified with a maximum of one intermediate gene from the correlating genes list. Orphan genes with no neighbours were removed from the network. The transcriptomics data analyses were

performed using the Anduril data analysis framework (433) and R (The R Foundation, R Development Core Team).

In article II, the bioinformatics analyses were done with GeneSpring GX 7.3 software. Based on the GO enrichment analysis, six pathways of interest—'branched-chain amino acid (BCAA) catabolism' (gene ontology [GO]: 009083), 'fatty acid β oxidation' (GO: 0006635), 'oxidative phosphorylation' (GO: 0006119), 'triacylglycerol synthesis' (GO:0019432), 'white adipose cell differentiation' (GO: 0050872) and 'chronic inflammatory response, CIRP' (GO: 0002544)—were selected, for which a mean centroid value (indicating mean activity of the pathway) was calculated. The mean centroid values for articles II, III and IV were calculated by normalizing the expression levels of the regulated genes in each pathway to a mean of zero and a variance of 1 across all individuals (262) and by using Affymetrix gene expression data for the genes included in the pathway. In addition, in articles III and IV, the mean centroid values were calculated for the expression of the small (MRPS, n = 30 (adipose tissue) /29 (adipocytes) subunits) and large (MRPL, n = 50 (adipose tissue) /49 (adipocytes) subunits) mitochondrial ribosomal proteins in adipose tissue and adipocytes respectively.

In articles III and IV of the thesis, the Limma (434) package of Bioconductor (429) was used to identify differentially expressed genes genome-wide between the obese and the lean co-twins in the discordant pairs. In the third article, we then looked whether the differentially expressed genes were found in MitoCarta (75), an online atlas of 1013 human genes, which encode proteins with mitochondrial localization. Forty-nine of the 1013 Mitocarta protein transcripts were not detectable by Affymetrix probes, including the 13 mtDNA-encoded OXPHOS transcripts. Identified mitochondria-related transcripts were further subjected to QIAGEN´s Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity) to reveal the pathways and the upstream regulators of the transcripts. The top 10 significant pathways were selected for further examination and for calculation of overall pathway activity. In article IV, the significantly differentially expressed genes between the co-twins in adipocytes and adipose tissue were subjected to IPA-analysis as above and the 10 top significant pathways and their mean centroids analyzed from both datasets. In addition, all the significantly differentially expressed genes between the co-twins that were also consistently up- or downregulated in the heavy co-twin, in at least 12 out of the altogether 14 discordant twin pairs were analyzed with IPA pathway analysis as above.

5.7. DNA methylation analyses (AT)

DNA methylation values in article III (beta-mixture quantile normalization, BMIQ) of all probes mapping to the differentially expressed MitoCarta genes and the upstream regulator PGC-1α in SAT were picked from the Illumina HumanMethylation BeadChip. Differential methylation analysis of the CpG sites on these genes was performed by using paired moderated t-tests (R Bioconductor) (429) in the discordant pairs and relationships between methylation in significant CpG sites and the expression of their associated genes by Spearman correlations in the individual twins.

5.8. Adipose tissue DNA extraction and qPCR for the measurement of mtDNA copy-number (AT)

Total DNA from the SAT samples of 15 BMI-discordant twin pairs was extracted with the standard phenol/chloroform and ethanol precipitation method. The amount of mtDNA was determined by a quantitative real-time PCR (qPCR). The amplification of mitochondrial cytochrome B (CYTB) gene was normalized to the amplification profile of nuclear amyloid beta precursor protein (APP) gene. The qPCR was performed from 25ng of total DNA by SYBR Green PCR Master Mix (iQ Custom SYBR Green Supermix, Bio-Rad, US) according to the manufacturer´s instructions on CFX96 Real-Time PCR Detection System (Bio-Rad). The PCR cycling conditions were: 95°C for 5min, followed by 40 cycles of 95°C for 15s, 60°C for 30s. Q-PCR data were analyzed by comparative ΔΔ Ct with Bio-Rad CFX Manager v.1.6 software.

5.9. qRT-PCR for the measurement of mtDNA transcripts (AT and adipocytes), mitochondrial regulator PGC-1α (AT and adipocytes) and other individual transcript levels (AT)

With AT samples, total extracted RNA (250ng) from SAT of 15 discordant twin pairs was treated with RQ1 DNase in 20µl of reaction volume (Promega, Madison WI, USA). cDNA was generated by Maxima synthesis kit (Maxima First Strand cDNA synthesis kit for qRT-PCR, Thermo Scientific) of DNAse treated RNA, done according to the manufacturer's instructions. PCR amplifications of mtDNA-encoded 12S rRNA (MT-RNR1), 16S rRNA (MT-RNR2), and mRNAs (MT-COX1, MT-ND5, MT-CYTB) were performed in 1/ 100 cDNA dilution with SYBRgreen PCR Master Mix (iQ Custom SYBR Green Supermix, Bio-Rad, US) according to the manufacturer´s instructions using CFX96 Real-Time PCR Detection System (Bio-Rad). The PCR cycling conditions were: 95°C for 5min, followed by 40 cycles of 95°C for 15s, 60°C for 30s. All samples were run in triplicates and data were analyzed with ΔΔ Ct method. The multicopy nuclear-encoded 18S rRNA gene and the two-copy GAPDH gene (data not shown) were used as control genes.

In adipocytes, a total of 300 ng of RNA was used to generate cDNA with the iScript cDNA Synthesis Kit (iScript cDNA Synthesis Kit, 170-8891, Biorad, UK) according to the manufacturer's instructions. PCR amplifications of mtDNA-encoded 12S rRNA (MT-RNR1), 16S rRNA (MT-RNR2), and mRNAs (MT-COX1, MT-ND5, and MT-CYTB) were performed with SYBRgreen PCR Master Mix according to the manufacturer's guidelines in the final volume of 20µl (IQ Custom SYBR Green Supermix 170-8860, Biorad, UK) by CFX96 Real-Time PCR Detection System (Bio-Rad). The PCR cycling conditions were as described above. The multicopy nuclear-encoded 18S rRNA gene, the two-copy GAPDH gene, YWHAZ gene and IPO8 gene were used as control genes. The qRT-PCR data were analyzed by qBASE+ (version 2.6.1, Biogazelle, Belgium) software, comparing the expression of target genes individually to the expression of all four control genes.

The Affymetrix microarray PGC-1α probes in AT and adipocytes were targeted to 3´UTR region of the longest mRNA isoform (ENSG00000109819) that is not shared with other protein coding PGC-1α isoforms. The expression of PGC-1α was validated by using iQ Real-Time PCR Detection

System (Bio-Rad) and the comparative ΔΔ Ct method with *18S rRNA* as a housekeeping gene for AT (article III). For adipocytes (article IV) the data were analyzed by qBASE+ (version 2.6.1, Biogazelle, Belgium) software as described above with four individual control genes. The target genes of PGC1α- and other upstream regulators were found in the upstream regulator analysis of IPA.

5.10. Western Blot for the measurement of OXPHOS protein levels (AT and adipocytes)

For article III, SAT from seven discordant twin pairs was available for protein extraction. Sample lysates were obtained by homogenization in 1% n-dodecyl β-D-maltoside (DDM, Cayman) in PBS with protease inhibitors (Roche). For the Western Blot, 20µg of total adipose tissue lysates was separated on 12% SDS-PAGE gels. The primary antibodies used were mouse monoclonal antibodies against complex I – NDFUA9 (MS111, Mitosciences), complex II 70kDa subunit (MS204, Mitosciences), complex III core 2 subunit (MS304, Mitosciences), complex IV subunit I (MS404, Mitosciences), and complex V subunit α (MS507, Mitosciences), The rabbit polyclonal antibodies were against PORIN (Abcam) and β-TUBULIN (Cell Signaling), and goat polyclonal antibody against ACTIN (I-19, Santa Cruz Biotechnology). Antibodies were diluted in 1% BSA/ Tris-buffered saline and 0.1% Tween 20 (TBST) at 1:2000 (CI), 1:5000 (CII), 1:2500 (CIII), 1:500 (CIV) and 1:1000 (PORIN, TUBULIN, ACTIN) and incubated at 4°C overnight. Secondary HRP-conjugated anti-mouse, anti-rabbit and anti-goat antibodies (1:10 000, Molecular Probes) were incubated with the membranes in 1% milk/ TBST for 1h at room temperature. The proteins were quantified with Image Lab v3.0 software.

For article IV, adipocytes from six obese and seven lean individual volunteers were used for Western Blot analysis. Protein lysates were obtained by homogenization in ice-cold RIPA buffer with protease inhibitors (Roche). For the Western Blot, 10µg of the lysates was separated on 4-20% SDS-PAGE gels. The primary antibodies used were Total OXPHOS antibody mixture (110411; Abcam); NDUFB8 (complex I), SDHB (complex II), subunit core 2 (UQCRC2) of complex III, COX2 (complex IV) and ATP5A (complex V). Additionally, individual antibodies were used for complex III core 2 subunit (MS304) and complex V subunit α (MS507, Mitosciences). Actin (Santa Cruz Biotechnology), Vinculin (Abcam ab129002) and Porin (Abcam) were used as controls. Antibodies of Total OXPHOS and Vinculin were incubated in 5% milk/ Tris-buffered saline and 0.1% Tween 20 (TBST) and CIII, CV, Actin and Porin in 1% BSA/TBST at 1:1000 concentrations overnight in 4°C. Vinculin was used at 1:10 000 concentration. Secondary HRP-conjugated anti-mouse (Santa Cruz), anti-rabbit (Jackson ImmunoResearch) and anti-goat (Calbiochem 401504) (1:2500) were used in 5% milk/TBST for 1h in room temperature. The proteins were quantified with ImageLab v3.0 software.

5.11. Family history and lifestyle factors (diet, alcohol intake, smoking and physical activity) in the discordant pairs

The dietary intake of the twins was assessed from three-day food records and analyzed by the Diet32 program (Aivo), based on a national Finnish database for food composition (Fineli, www.fineli.fi, National Institute for Health and Welfare, Nutrition Unit, Helsinki, Finland). Information on the weekly alcohol intake and smoking habits during the past four weeks before the study (study II) was obtained by structured questionnaires. Physical activity was assessed by Baecke- questionnaire and its three sub-compartments (sport-, leisure time and work indexes) (435). The parents were examined by questionnaires (age, height, weight) when the twins were 16 years of age.

5.12. Statistical analyses

Basic statistical analyses were performed using Stata statistical software (Releases 11.0, 12.0 and 13.0 Stata Corporation, College Station, TX). Results are expressed as mean±SE. Differences between co-twins were calculated by paired Wilcoxon's signed rank tests and differences in the protein levels between lean and obese individuals by Mann Mann–Whitney U -test. Intra-class correlations were used to analyze the resemblance between the co-twins. To test the frequencies between two groups, chi-squared distributions were exploited. Within pair differences (Δ) of all clinical and metabolic parameters were obtained by subtracting the leaner co-twin's value from the heavier co-twin's value. Within-pair differences (Δ) were then correlated using the Spearman correlations. QRT-PCR and Western Blot results were calculated by paired Wilcoxon's signed rank tests for twins. Correlations between metabolic and the investigated variables in all concordant and discordant twin individuals were calculated using Pearson correlations, corrected for clustered sampling of co-twins by survey methods (436). Logarithmic corrections (log base 10) were used for non-normally distributed variables, and the correlation coefficients adjusted for multiple comparisons. Since the high correlation among the variables made the traditional Bonferroni correction for multiple testing too conservative, the number of principal components was used to provide a proper P-value threshold. The variables in the correlation tables presented in the thesis produced one to five principal components, which explained 79-90% of the variance. Therefore, the adjusted significance level for analyses in the subsequent correlation tables were $P < 0.05$ divided by the number of principal components that were analyzed for each table separately. To test whether adipocyte volume and size were related to clinical and metabolic measures independent of the within pair difference in body fat, the ratios of Δadipocyte volume/Δkg fat and Δadipocyte number/Δkg fat were used in the correlation analyses. Multiple regression analysis was used to test whether Δadipocyte volume and Δadipocyte number independently associated with metabolic measures.

In article II, the obesity-discordant MZ pairs were divided into two subgroups according to the amount of their liver fat: those who were discordant - group 2 with high ≥2% within-pair differences in liver fat (n = 8) - and those who were concordant - group 1 with low <2% delta Δ liver fat (n = 8). In group 1 were those obesity-discordant co-twins that were metabolically similar, and in group 2 those co-twins where the obese co-twin had a higher liver fat amount compared to their leaner co-twins and metabolic disturbances. The Mann–Whitney U test was used to compare differences between lean in the liver fat group 1 vs group 2, as well as obese in group 1 vs group 2. Sex

distributions between the groups were tested by the $\chi 2$ test. Analyses on leptin were performed with sex-adjusted values because women had higher circulating and adipose tissue transcript levels of leptin than men. Multiple regression analyses were performed to detect independent predictors of Δliver fat.

GO enrichment analysis was used to compute hypothesis-free enrichment of GO terms (molecular function) in each of the groups in article II (weight-concordant, group 1 discordant and group 2 discordant). Enrichment analysis was done using Fisher's Exact Test. Fisher's test compares the observed frequency of each present GO term to the frequency in a reference gene set (whole gene array). A GO term is present if some input gene is annotated with the GO term or its descendants. The p-values were adjusted using the Benjamin and Hochberg FDR. The GO enrichment analysis was performed using the Anduril data analysis framework (433) and R.

Identical twins are a powerful study setting. Power calculations show that 12 twin pairs is needed to detect 20% differences with $P<0.05$ significance level and 0.80 power in insulin resistance and other studied metabolic measures between the obese and the lean co-twin.

6. RESULTS

6.1. Characteristics of the twins (I-IV)

Characteristics of the twins are presented in Table 1. In total, the obese (BMI 31.3 ± 1.0 kg/m^2) and the lean (25.3 ± 0.9 kg/m^2) co-twins of the discordant pairs (n = 26) had a mean difference of 18 ± 0.5 kg (P <0.001) in body weight. The obese co-twins had significantly more SAT, VAT, and liver fat; larger adipocytes; higher plasma leptin levels; lower plasma adiponectin levels; lower HDL and higher LDL cholesterol level as well as higher plasma triglyceride levels, and they were more insulin resistant than the lean co-twins. The concordant co-twins (n = 14) had similar body composition and metabolic measures (Table 1) and also did not differ in their mitochondrial parameters.

Table 1: Clinical characteristics of the monozygotic (MZ) twins

Variable	**Weight-discordant pairs** (ΔBMI >3 kg/m2, n = 26)			**Weight-concordant pairs** (ΔBMI <3 kg/m2, n = 14)
	Leaner co-twin	**Heavier co-twin**	**p-value***	**Both co-twins**
Age (years)	29.9 ± 0.9	29.9 ± 0.9	0.33	31.6 ± 0.6
Weight (kg)	75.4 ± 3.5	93.3 ± 4.0	< 0.0001	79.3 ± 2.5
Height (cm)	171.9 ± 2.0	172.2 ± 1.9	0.38	171.5 ± 2.0
BMI (kg/m2)	25.3 ± 0.9	31.3 ± 1.0	< 0.0001	26.9 ± 0.7
Body fat %	32.3 ± 1.8	41.1 ± 1.3	< 0.0001	29.2 ± 1.7
Fat (kg)	24.9 ± 2.2	38.2 ± 2.1	< 0.0001	23.5 ± 1.7
Fat free mass (kg)	48.0 ± 2.1	52.1 ± 2.5	0.0001	53.4 ± 2.0
Subcutaneous fat (dm^3)	3813.7 ± 416.8	6358.9 ± 540.4	< 0.0001	3256.3 ± 261.2
Intra-abdominal fat (dm^3)	790.2 ± 178.9	1643.7 ± 247.4	< 0.0001	1065.2 ± 130.0
Liver fat (%)	1.12 ± 0.32	4.52 ± 0.99	< 0.0001	2.87 ± 0.98
Adipocyte volume (pL)	355.6 ± 34	547 ± 59	0.0008	412.0 ± 46.2
Adipocyte number (10^{13})	8.4 ± 0.74	8.3 ± 0.60	0.95	7.82 ± 0.60
fP-glucose (mmol/L)†	5.1 ± 0.1	5.3 ± 0.1	0.17	5.4 ± 0.1
fP-insulin (mU/L)†	4.9 ± 0.5	8.5 ± 1.2	0.0011	5.5 ± 0.6
HOMA-index†	1.1 ± 0.1	2.1 ± 0.3	0.0010	1.3 ± 0.2
Matsuda-index†	8.6 ± 0.9	6.0 ± 0.7	0.0089	9.9 ± 1.3
AUC Insulin in OGTT (mU/L)	87.6 ± 8.0	129.3 ± 24.6	0.031	77.5 ± 10.9
Total cholesterol (mmol/L)	4.4 ± 0.2	4.7 ± 0.2	0.14	4.5 ± 0.2
LDL cholesterol (mmol/L)	2.6 ± 0.1	3.0 ± 0.2	0.034	2.8 ± 0.2

HDL cholesterol (mmol/L)	1.6 ± 0.1	1.3 ± 0.1	0.0004	1.3 ± 0.1
Triglycerides (mmol/L)	0.94 ± 0.1	1.32 ± 0.2	0.014	1.02 ± 0.13
fP-Leptin (mg/mL)	18.9 ± 4.1	34.6 ± 5.5	0.0015	28.4 ± 10.2
fP-Adiponectin (µg/mL)	2.8 ± 0.3	2.2 ± 0.2	0.0023	2.2 ± 0.1
fS-hs-CRP (mg/dL)	2.6 ± 0.7	4.0 ± 1.1	0.065	1.2 ± 0.3
Adipsin (ng/mL)	1190 ± 45	1310 ± 47	0.0063	1070 ± 71
Resistin (ng/mL)	9560 ± 1440	10060 ± 1360	0.0694	7670 ± 4630
Total physical activity	8.9 ± 0.2	8.3 ± 0.2	0.213	8.9 ± 0.3
Sport-index	3.2 ±0.2	2.7 ± 0.2	0.061	3.3 ± 0.2
Leisure-index	2.8 ± 0.1	2.8 ± 0.1	0.837	2.8 ± 0.1
Work-index	2.8 ± 0.1	2.8 ± 0.1	0.466	2.8 ± 0.1

*Wilcoxon's rank sum test was used to compare values of the leaner versus the heavier co-twin. †*n* = 25 obesity-discordant MZ pairs. AUC, area under the curve; fP, fasting plasma; fS, fasting serum; HOMA, homeostatic model assessment; hs-CRP, high-sensitivity C-reactive protein; OGTT, oral glucose tolerance test; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue. Data represent means with SE.

6.2. Adipose hypertrophy and hyperplasia in obesity (I)

6.2.1 Obesity increases adipocyte hypertrophy, but adipocyte number remains the same due to genetic factors

Adipocyte size is known to increase in obesity, but how this is influenced by genetic factors has been debated. Of the total 40 twin pairs included in this thesis, study I involved 20 pairs of MZ twins (ΔBMI range: 0.86-9.95 kg/m2), of which 15 were defined as discordant for obesity (ΔBMI > 3kg/m^2, here mean±SE ΔBMI 5.7±0.55) and the other 5 were defined as concordant (ΔBMI <3kg/m^2, here mean±SE ΔBMI 1.7±0.37) (Table 1). The concordant MZ pairs were remarkably similar both for adipocyte number (intra-class correlation between the obese and the lean co-twin ICC=0.91, p=0.0021, 95%CI 0.77-1.06) and for adipocyte size (ICC=0.92, p=0.0021, 95%CI 0.79-1.05) (Figure 10). However, for the discordant MZ pairs, the twins resembled each other for adipocyte number (ICC=0.63, p=0.0041, 95%CI 0.31-0.94) but less so for size (ICC=0.34, p=0.094, 95%CI 0.00-0.80). Each individual presented with a wide distribution of cell sizes. In the discordant pairs, the distribution of adipocyte cell size differed significantly between the co-twins (Figure 10). When compared with the lean co-twins, the obese had significantly fewer small cells (volume<160 pL) (20% vs. 31%, p=0.0046) and more extra-large cells (volume 550-2,000 pL) (29% vs. 21%, p=0.039). The differences in the proportions of medium-sized (volume 160-330 pL) or large cells (330-550 pL) were not significant between obese and lean twin pair members (23% vs. 27%, p=0.15, and 27% vs. 21%, p= 0.075). On average, the cell size was larger in the obese than in the lean co-twins (61±12%, range -2.9-122%, p=0.0008) (Table 1). All but one of the obese co-twins had larger mean adipocyte size compared with the lean co-twins.

Figure 10: Adipocyte volume and number in the discordant and concordant co-twins

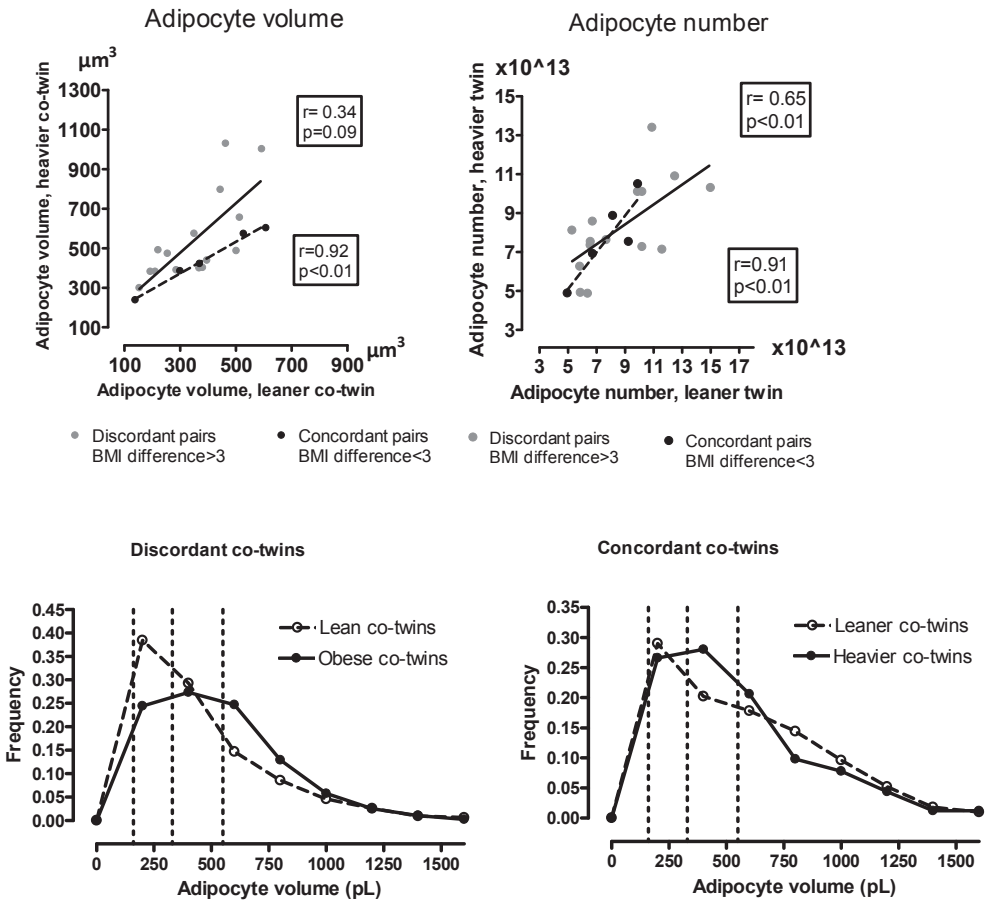


Figure 10: *In the concordant co-twins the within-pair differences (intra-class correlation, ICC) in both adipocyte volume ($r=0.92$, $p \leq 0.01$) and number ($r=0.91$, $p<0.01$) were linearly correlated between the co-twins, speaking for a tight regulation of adipocyte number and volume. The distribution of small and large cells did not differ significantly between the co-twins. In the discordant pairs, adipocyte number increased linearly ($r=0.65$, $p<0.01$) between the twins, but adipocyte volume less so ($r=0.34$, $p=0.09$). During weight gain, adipocyte number seems to be stable and based on the extreme similarity between the MZ twins, being tightly genetically regulated, while environmental factors affect adipocyte volume in an excess energy state. In the discordant pairs, the lean co-twins had significantly more small adipocytes ($p=0.0046$) and less extra-large cells ($p=0.039$) than their obese peers.*

When inspecting the obesity-discordant data as a whole, the total cell numbers did not differ between the co-twins (Table 1), but in turn, considerable variability between twin pairs could be observed. In a proportion (7/15) of the discordant twin pairs the obese co-twins had more while in the other proportion (8/15) they had less adipocytes than their leaner co-twins. The range of Δadipocyte number (obese minus lean co-twin) varied from -38% to 53%. This suggested that some twin pairs seemed to react to obesity with increased adipose cell proliferation and thus hyperplasia, while others reacted with more pronounced hypertrophy (and hypoplasia). Obese co-twins who had an increased adipocyte count compared with their lean co-twins (hyperplastic obesity) were indistinguishable from their lean counterparts in most metabolic measurements (Figure 11). In contrast, obese co-twins who had a more hypertrophic obesity profile with a decreased cell count compared with their leaner co-twins had significantly more liver fat, insulin resistance, inflammation and LDL cholesterol than the lean twin pair members (Figure 11). According to these results it would seem that the larger the capacity for hyperplasia in adipose tissue, the better the metabolic complications of obesity are prevented. The remarkable similarity between the concordant co-twins suggest that adipocyte phenotype is genetic or due to shared environmental factors.

Figure 11: Hypertrophic and hyperplastic obesity groups

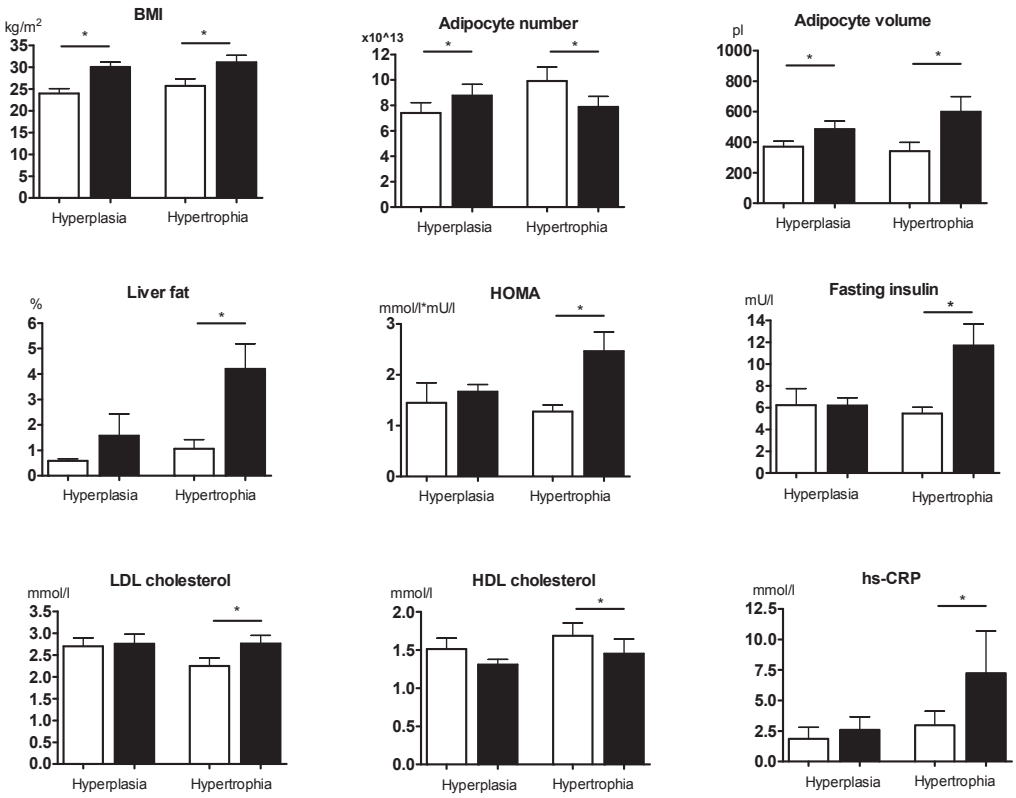


Figure 11: *The 15 obesity-discordant twin pairs could be divided into two metabolically different subgroups: the "hyperplastic" group (n=7 pairs), where the obese twins had more adipocytes than their leaner counterparts and the "hypertrophic" or "hypoplastic" group (n=8 pairs), where the obese co-twins had similar or even smaller total number of adipocytes than their leaner co-twins. Lean twins= white bars, obese twins= black bars. In the hypertrophic group, the obese twins had larger adipocyte volume, more liver fat, higher HOMA-index and fasting insulin concentrations as well as higher LDL cholesterol, hs-CRP and lower HDL cholesterol concentrations than their leaner co-twins. In the hyperplastic group, despite larger adipocyte volume than their leaner co-twins, the obese co-twins had similar amounts of liver fat and similar metabolic measurements compared to their leaner co-twins. Wilcoxon's rank sum test was used to compare values of the leaner vs. the heavier co-twin. *P<0.05. Data is presented as mean ± standard error.*

6.2.2 Adipocyte size but not number correlates with metabolic measures of obesity and insulin resistance

ΔAdipocyte size (obese minus lean co-twin) correlated positively with many of the metabolic measurements (Table 2, Article I). The correlations with Δadipocyte number however were more modest, and when present, to an opposite direction from those of Δadipocyte size. ΔAdipocyte volume correlated positively and Δcell number negatively with ΔHOMA index, Δinsulin, Δtotal cholesterol, ΔLDL and Δleptin and Δadipocyte size negatively with Δadiponectin. When Δadipocyte volume and Δadipocyte number were analysed in the same multiple regression model, the following associations were found for the metabolic measurements: Δadipocyte volume was independently associated with Δfasting insulin (β=0.017, SE=0.0076, p=0.042), ΔLDL cholesterol (β=0.0018, SE=0.00081, p=0.043), Δtriglycerides (β=0.019, SE=0.00060, p=0.005), Δleptin (β=53.0, SE=21.4, p=0.028), and Δadiponectin (β=-2.6, SE=0.98, p=0.021). Additionally Δadipocyte number but not Δadipocyte volume was inversely associated with ΔCRP (β=-1.8e-13, SE=5.5e-14, p=0.006). Results are presented in Table 2, article I. Independent of the degree of body fat difference between the co-twins, Δfasting serum insulin was associated with increased Δadipocyte volume/Δkg fat (r=0.66, p=0.0023) and reduced Δadipocyte number/Δkg fat (r= -0.72, p=0.0005). Also ΔHOMA-index was associated with increased Δadipocyte volume/Δkg fat (r=0.72, p=0.0005) and reduced Δadipocyte number/Δkg fat (r= -0.65, p=0.0026). ΔLDL cholesterol correlated positively with Δadipocyte volume/Δkg fat (r=0.57, p=0.0094) and negatively with Δadipocyte number/Δkg fat (r=-0.53, p=0.0154) as did also Δtotal cholesterol/Δkg fat (r=0.48, p=0.0326), (r=-0.47, p=0.0365). Δadipocyte volume/Δkg fat was related to Δleptin/Δkg (r=-0.50, p=0.0471). The other associations between adipocyte morphology and metabolism were not significant after adjusting for Δbody fat.

6.2.3 Gene expression associated with increased adipocyte size reveals mitochondria- and cell death-related pathways, correlating with metabolic measures of the body

To shed light on the genes and pathways behind increased adipocyte hypertrophy, we linked obesity-associated changes in adipocyte size and number with global adipose tissue gene expression. We set out to find genes where the gene expression fold changes (heavier/leaner co-twins in all pairs) correlated most positively with fold change (heavier/leaner co-twins) in adipocyte size. Correlation limit was set to 0.6 and the FDR-corrected p-Value was 0.25. To find out the networks that would be behind increased adipocyte size, we generated an interaction map with Moksiskaan analysis of those genes, that were significantly positively or negatively associated with adipocyte size (simplified in Figure 12). The genes were clustered into six interconnected entities which we named: i) mitochondrial function (cluster 1); ii) lipid synthesis and modification of lipid peroxidation products (cluster 2); iii) aldehyde-, ketone- and steroid metabolism, and modification of the cell membrane (e.g. releasing arachidonic acid) (cluster 3); iv) RNA transcription as well as DNA packaging and repair (cluster 4); v) ubiquitination of target proteins, DNA-damage and cell cycle changes, especially in the G1/S transition phase (cluster 5); and vi) tumour growth factor β (TGF-β) signaling, programmed cell death, p53 pathway and proapoptosis (cluster 6).

Figure 12: Interaction map of the genes associating to adipocyte size

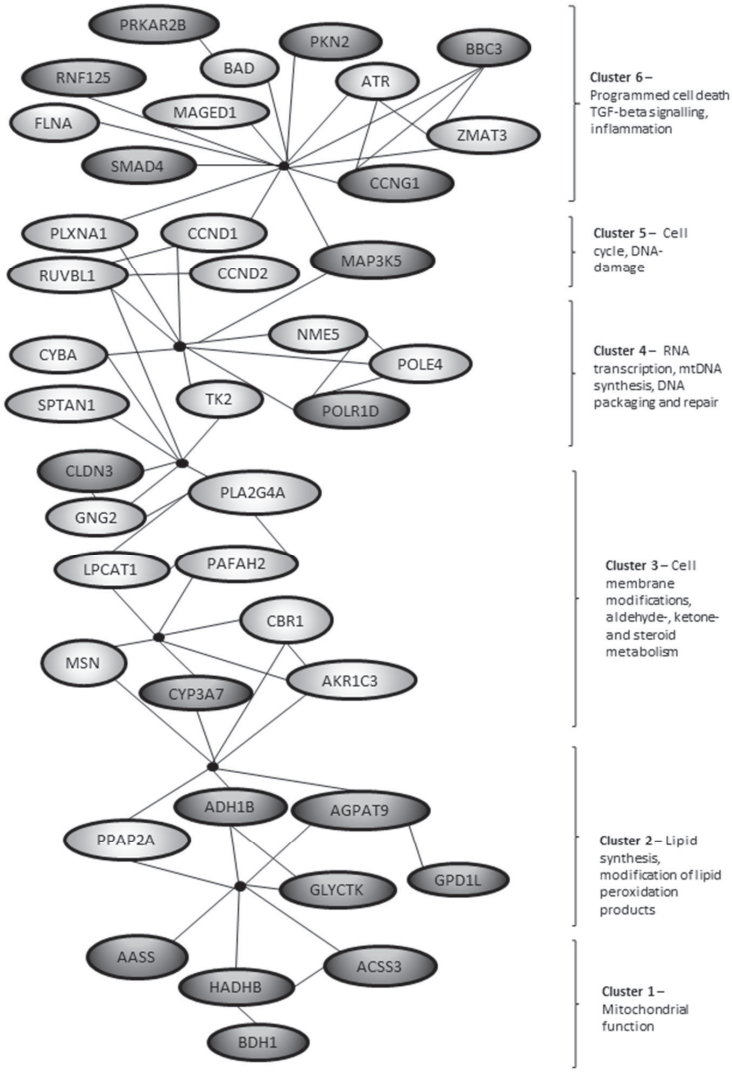


Figure 12: *Gene networks associated with adipocyte volume within 20 monozygotic twin pairs (fold change of heavier/leaner co-twins for adipocyte volume and for each gene), generated by Moksiskaan database analysis. Six gene clusters intertwined to each other emerged in the analysis. Fold change of genes correlating positively with fold change in adipocyte size are presented as light grey and fold change of genes correlating negatively with fold change in adipocyte size as dark grey.*

A heatmap of the expressions of the genes in these clusters with clinical phenotypes (Article I, Figure 4) illustrates that transcripts associated with mitochondrial function (cluster 1) correlated significantly negatively with adipocyte size, subcutaneous, visceral and overall body fat measurements, fasting insulin and leptin. The same was true for triglyceride synthesis and glycerol metabolism associated transcripts (cluster 2) except for the gene PPAP2A, for which the correlations were opposite. Transcripts involved in modifications and oxidoreduction of aldehydes, ketones and lipids, cell membrane associated genes (cluster 3), genes of DNA repair, and packaging (cluster 4), cell cycle arrest (cluster 5), cell death and inflammation (cluster 6) correlated positively with adipocyte size, body fat, fasting insulin and leptin.

6.3 Metabolically healthy and unhealthy obesity (II)

6.3.1 Two distinct metabolic groups of obesity-discordant MZ pairs

A completely novel finding in the second study of the thesis was that the obesity-discordant MZ twin pairs could be divided into two distinct metabolic groups according to the amount of their liver fat. In half of the pairs (group 1, n=8), the obese co-twins had as low liver fat percentages as their leaner co-twin (Δ liver fat = 8%, p=0.21), whereas in the other group (group 2, n=8) the obese co-twins had a strikingly increased liver fat content compared with their leaner co-twins (Δ liver fat = 718%, p = 0.012) (Figure 13). These two groups did not differ in the measure overall fatness (weight, BMI, fat kg, fat %, SAT), but group 2 had increased ΔVAT content compared with group 1 (Figure 15 b, c). In group 1, there were no differences in the insulin or glucose curves during OGTT, and no differences in HOMA-index, Matsuda-index or circulating lipids (LDL, HDL) between the co-twins. However in group 2, the obese co-twins had significantly higher AUC for glucose (23%, p = 0.028) and insulin (78%, p = 0.028) during the OGTT (Figure 14, 119%, p = 0.018), higher HOMA-index and 55% (p = 0.028) lower Matsuda index (Figure 15 d, e) as well as significantly higher LDL- and lower HDL-cholesterol levels (Table 1, Article II) than their leaner co-twins.

Figure 13: Division of twins into two subgroups of obesity according to the amount of liver fat in the obese co-twins

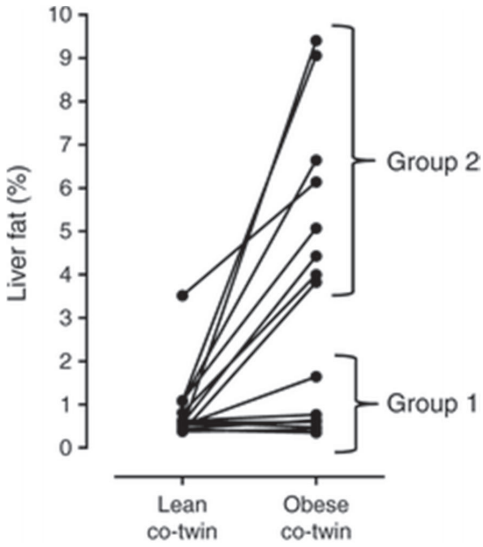


Figure 13: *The obese and the lean co-twins could be divided into two subgroups of obesity, according to the amount of liver fat in the obese co-twin. In group 1 (n=8 pairs, liver fat % ≤ 2), the obese co-twins were very similar to their leaner counterparts in the liver fat measures. In group 2 (n=8, liver fat % ≥2), the obese co-twins had significantly increased liver fat amounts compared to the lean co-twins.*

Figure 14: Glucose and insulin during the OGTT (oral glucose tolerance test) in the obese and lean twins of groups 1 and 2

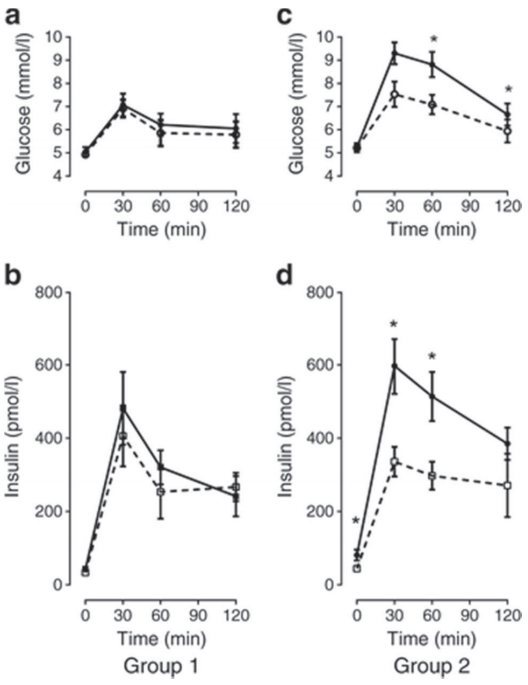


Figure 14: *Glucose (a) and insulin (b) during the OGTT in group 1. Glucose (c) and insulin (d) during the OGTT in group 2. Obese twins in group 2 presented with significantly augmented responses compared with their leaner co-twins in both glucose and insulin during the test.*

Figure 15: Metabolic characteristics of the twins divided in two subgroups on the basis of their liver fat content

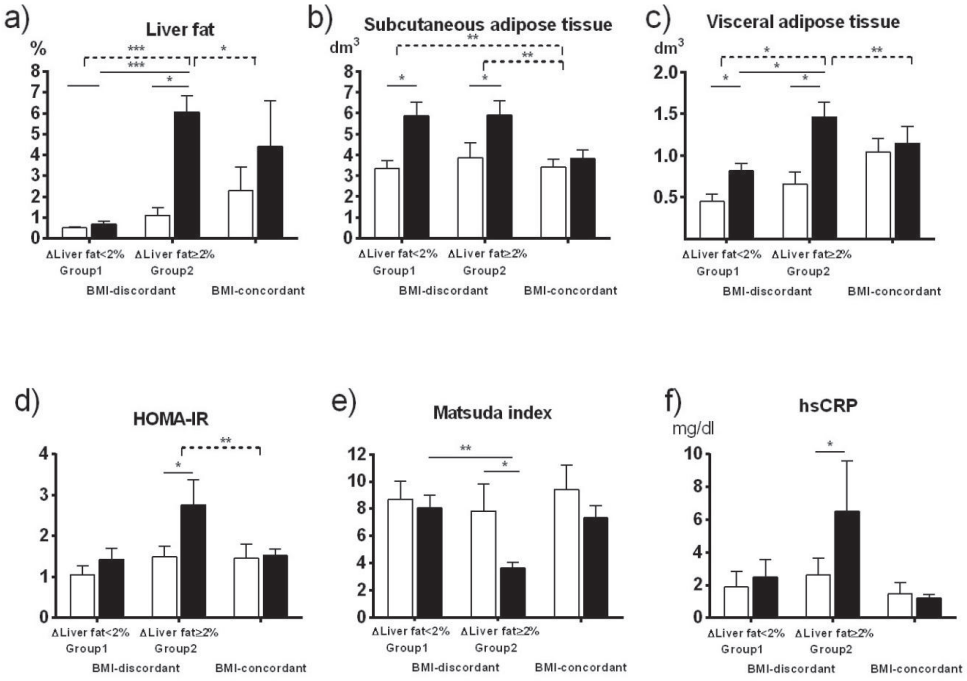


Figure 15: *Metabolic characteristics of the BMI-discordant co-twins stratified into two subgroups according to the amount of liver fat in the obese co-twin. White bars present lean twins and black bars obese twins. BMI-concordant pairs are presented as reference. ***P<0.001, **P<0.01, *P<0.05. Data is presented as mean ± SE. In group 1 (n=8 twin pairs) the obese co-twins did not significantly differ from their leaner counterparts in their metabolic measures. In group 2 (n=8), the obese twins had more liver fat, subcutaneous and visceral fat, higher hs-CRP levels and were more insulin resistant than their leaner co-twins.*

The mean adipocyte cell size was increased in obese co-twins of both groups, but Δadipocyte number differed between the groups (p = 0.037). In group 1 the obese co-twins had more adipocytes than their leaner counterparts (11%, p = 0.069), whereas in group 2, the obese co-twins had even less adipocytes (8%, p = 0.13) than their lean co-twins. Interestingly, the result of two different groups of adipocyte hyperplasia or hypertrophy was reported in article I, where the twins divided in the hyperplastic group had insignificant difference in their liver fat between the obese and the lean co-twin, while in the hypertrophic group, there was a significant difference in the liver fat profiles of the co-twins, the heavier co-twins having much more liver fat than their leaner counterparts.

6.3.2. Distinct gene expression profiles in the two metabolically different obesity groups

When the gene expression profiles of the two groups were compared, in group 2 the obese and the lean co-twins differed significantly for mitochondrial pathways. 'Oxidoreductase activity' (GO: 0016491) (FDR corrected $p = 0.045$) and 'Cofactor binding' (GO: 0048037) ($p = 0.0096$), the latter of which also included several mitochondrial genes, were lower in the obese co-twins and restricted to group 2. In group 1, the obese and lean co-twins differed only for 'structural constituent of ribosome' (GO: 0003735) (FDR corrected $p = 0.016$). Based on these findings, additional pathways were analyzed and three mitochondrial pathways selected to represent different aspects of energy handling; oxidative phosphorylation pathway ($p = 0.48$ in group 1, $p = 0.028$ in group 2) (Figure 16a); BCAA catabolism pathway ($p = 0.48$ in group 1, $p = 0.018$ in group 2) (Figure 16b) and fatty acid β oxidation pathway ($p = 1.0$ in group1, $p = 0.018$ in group 2) (Figure 16c) were all significantly different and downregulated in the obese co-twins compared with their lean twins in SAT of group 2 but not group 1. Also, the activity of the chronic inflammatory response pathway (CIRP) was observed to be significantly elevated in the obese co-twins of group 2 ($p = 0.028$) but not group 1 ($p = 0.31$). (Figure 16f) Two pathways representing SAT enlargement were also analyzed (triacylglycerol synthesis and adipocyte cell differentiation). The triacylglycerol synthesis pathways did not differ between the obese and lean co-twins' SAT in either of the groups (Figure 16e). In contrast, the adipocyte differentiation pathway was significantly downregulated in group 2 obese co-twins ($p = 0.004$), whereas the expression pathways in group 1 co-twins were similar ($p = 0.33$), (Figure 16d).

Figure 16: Gene expression profiles of twins from the two metabolically different subgroups

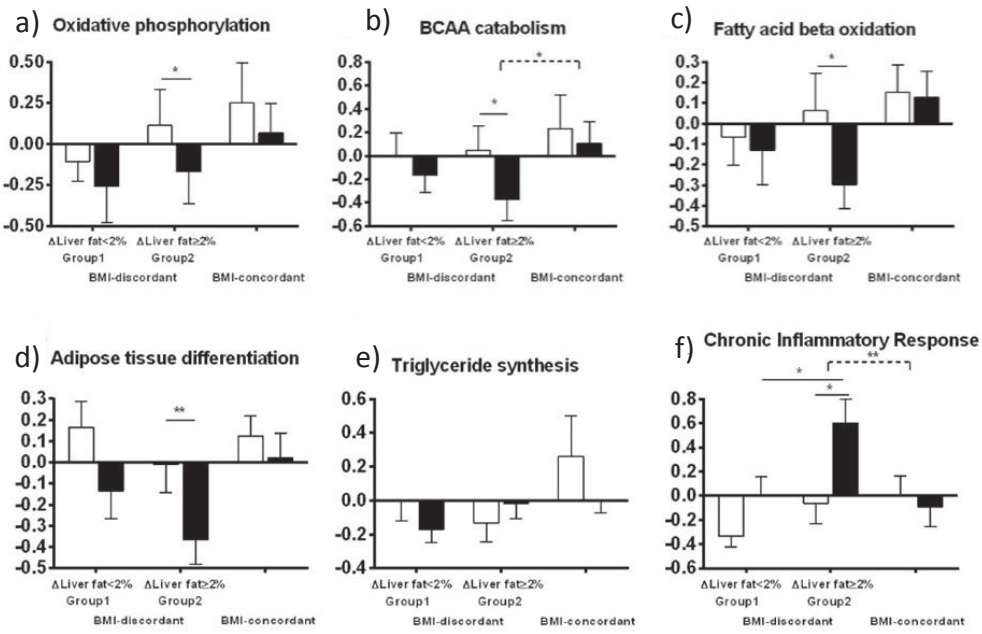


Figure 16: *Gene expression profiles of the two metabolically different subgroups in twins, separated according to the amount of liver fat in the obese co-twin. White bars represent lean twins and black bars obese twins. BMI-concordant pairs are presented as reference. **P<0.01, *P<0.05. Expression values represent the mean activation of the pathway by mean centroid values. Error bars indicate standard error. Oxidative phosphorylation, fatty-acid beta oxidation, BCAA catabolism and adipose tissue differentiation pathways were downregulated and chronic inflammatory response pathway (CIRP) upregulated in the obese twins of the group 2 with increased liver fat content in the obese co-twin.*

6.4 Downregulation of mitochondrial biogenesis in obese adipose tissue (III)

6.4.1. Downregulation of mitochondria-related transcripts and pathways in obese adipose tissue and differential methylation between the obese and the lean co-twins

Due to the mitochondria-related results in the previous two articles, in the third article of the thesis (III) we set out to investigate the transcriptomic profile of adipose tissue by concentrating on the mitochondria. In this article, we had the full 26 discordant and 14 concordant MZ twin pairs in use for the transcriptomics data analyses. With these analyses we found that there were many mitochondria-related genes and pathways in the significantly differentially expressed genes between the co-twins. We first investigated the within-pair differences in the expression of mitochondrial proteins in SAT (n = 26 twin pairs) by linking the results from the significantly differentially expressed genes between the co-twins from transcriptomics data with the genes of MitoCarta. Among the 2108 significantly differentially expressed genes, 222 genes were listed in MitoCarta (Supplementary data S1). Most of these transcripts (187 of 222, 84%) were downregulated in the obese compared with the lean co-twins´ SAT.

We then investigated which functional entities of the mitochondrial proteins the 222 transcripts represented. We subjected the transcripts to IPA (Ingenuity Pathway Analysis) and found out that among the most significantly different and downregulated pathways in the obese compared with the lean co-twins were the key mitochondrial functions: OXPHOS ($p < 0.0001$), BCAA degradation ($p < 0.0001$), ketogenesis/ketolysis ($p < 0.0001$), the tricarboxylic acid cycle (TCA, $p < 0.0001$), glutaryl-CoA degradation ($p < 0.0001$), and fatty acid β-oxidation (FAO, $p < 0.0001$; Table 2). We correlated the calculated mean centroid values (obtained by normalizing the expression levels from Affymetrix data of the regulated genes in each pathway to a mean of zero and a variance of 1 across all individuals and thus obtaining a value representing the activity of the pathway) of the most significant pathways to measures of adiposity (SAT, VAT and liver fat, adipocyte volume), insulin sensitivity (Matsuda index), insulin resistance (HOMA index), inflammation (adipsin, hs-CRP), leptin and adiponectin. The mean centroid values of OXPHOS and TCA cycle pathways correlated negatively with SAT, VAT, liver fat, adipocyte volume, HOMA- index, adipsin and hs-CRP (Table 3) and positively with Matsuda-index and adiponectin. Glutaryl-CoA degradation, ketogenesis, and tryptophan degradation pathways correlated negatively with all of the selected clinical variables and positively with Matsuda-index and adiponectin. BCAA degradation (valine-, isoleucine-, and leucine degradation pathways), FAO, and ketolysis correlated negatively with all clinical variables, except positively with adiponectin and Matsuda-index.

Table 2: Pathways significantly changed in the obese compared with the lean co-twin
(IPA analysis of the differentially expressed genes, $n = 26$ discordant pairs)

Ingenuity canonical pathways	***P* value**	**Regulation**
Oxidative phosphorylation	< 0.0001	Downregulated
Glutaryl-CoA degradation	< 0.0001	Downregulated
Isoleucine degradation I	< 0.0001	Downregulated
Ketogenesis	< 0.0001	Downregulated
Fatty acid β-oxidation I	< 0.0001	Downregulated
Tryptophan degradation III (eukaryotic)	< 0.0001	Downregulated
Ketolysis	< 0.0001	Downregulated
TCA cycle II (eukaryotic)	< 0.0001	Downregulated
Leucine degradation I	< 0.0001	Downregulated

IPA, Ingenuity Pathway Analysis

Table 3A: Correlations of mitochondrial variables with metabolic measures of individual MZ twins (n = 80)

Variable	**SAT**	**VAT**	**Liver fat**	**Cell volume**	**Matsuda**
mtDNA	-0.2544	-0.0835	-0.1761	-0.2076	0.2954
Oxidative phosph.	-0.367†	-0.603‡	-0.420†	-0.497†	0.445‡
Fatty acid β-ox	-0.622‡	-0.669‡	-0.518‡	-0.584‡	0.669‡
TCA cycle	-0.315†	-0.660‡	-0.432†	-0.452†	0.483‡
Glutaryl-CoA degr.	-0.690‡	-0.644‡	-0.469‡	-0.586‡	0.634‡
Ketogenesis	-0.661‡	-0.652‡	-0.480‡	-0.569‡	0.623‡
Ketolysis	-0.657‡	-0.627‡	-0.478‡	-0.541†	0.626‡
Valine degr.	-0.585‡	-0.702‡	-0.559‡	-0.596‡	0.658‡
Leucine degr.	-0.430†	-0.649‡	-0.485‡	-0.489†	0.578‡
Isoleucine degr.	-0.533‡	-0.641‡	-0.472‡	-0.512‡	0.602‡
Tryptophan degr.	-0.630‡	-0.644‡	-0.469‡	-0.586‡	0.634‡

Table 3B: Correlations of mitochondrial variables with metabolic measures of individual MZ twins (n = 80)

Variable	HOMA	Leptin	Adipo nectin	hs-CRP	Adipsin
mtDNA	-0.4582*	-0.2413	-0.0408	-0.0166	-0.4007*
Oxidative phosph.	-0.322†	-0.191	0.353*	-0.384†	-0.3402*
Fatty acid β-ox	-0.595‡	-0.519‡	0.378†	-0.476‡	-0.4174†
TCA cycle	-0.388‡	-0.147	0.456‡	-0.416‡	-0.4241†
Glutaryl-CoA degr.	-0.558‡	-0.543‡	0.330*	-0.470‡	-0.4484‡
Ketogenesis	-0.570‡	-0.488†	0.358*	-0.437†	-0.4337‡
Ketolysis	-0.587‡	-0.490†	0.387*	-0.437†	-0.4671‡
Valine degr.	-0.638‡	-0.425†	0.524‡	-0.486‡	-0.5377‡
Leucine degr.	-0.506‡	-0.306†	0.411†	-0.412‡	-0.4003†
Isoleucine degr.	-0.581‡	-0.391*	0.486‡	-0.430‡	-0.5187‡
Tryptophan degr.	-0.558‡	-0.543‡	0.330*	-0.470‡	-0.4484‡

*P < 0.05, †P < 0.01, ‡P < 0.001. fP, fasting plasma; HOMA, homeostatic model assessment; hs-CRP, high-sensitivity C-reactive protein; SAT, subcutaneous adipose tissue; TCA, tricarboxylic acid cycle; VAT, visceral adipose tissue.

6.4.2. Reduced mtDNA amount, mtDNA encoded transcripts, mitochondrial ribosomal protein subunits and OXPHOS protein levels in the adipose tissue of the obese co-twins

As the mitochondrial transcripts and pathways dominated the above transcriptomics analyses, we then further investigated the effect of obesity on mitochondrial amount and biogenesis in SAT, by measuring the amount of mtDNA and the expression of mtDNA-encoded genes (12S rRNA, 16S rRNA, *COX1, ND5,* and *CYTB*, n = 15 discordant pairs) as well as Affymetrix gene expression of mitochondrial ribosomal proteins that translate the mitochondrial transcripts into proteins. The mtDNA amount was reduced by ~20% ($p = 0.031$, Figure 17B) in SAT of the obese compared with the lean co-twins. Significant negative correlations were observed between the mtDNA amount and HOMA (insulin resistance) and adipsin (inflammation) (Table 4). Mitochondrial 12S (*MT-RNR1*, $p = 0.0064$) and 16S (*MT-RNR2*, $p = 0.0090$) rRNAs, as well as mRNAs *COX1* ($p = 0.0064$, CIV subunit), *ND5* ($p = 0.027$, CI subunit), and *CYTB* ($p = 0.0015$, CIII subunit, Figure 17C), were decreased in SAT of the obese co-twins compared with their lean counterparts. We also studied the expression of 30 nuclear transcripts for the small subunit (MRPS) and 50 nuclear transcripts for the large subunit (MRPL) of the mitochondrial ribosome using the Affymetrix data (n = 26 discordant pairs). In line with 12S and 16S rRNA expression, the average value of the total expression (a mean centroid) of both MRPS (lean 0.03 ± 0.09 AU vs. obese -0.31 ± 0.1 AU, $p < 0.001$) and MRPL (lean -0.04 ± 0.1 AU vs. obese -0.26 ± 0.1 AU, $p = 0.0034$) was lower in the obese compared with the

lean co-twins (Figure 17D). The expression of the main mitochondrial regulator PGC-1α was reduced in the obese co-twins (qRT-PCR, $p = 0.004$, Figure 17A).

Finally, we measured mitochondrial content and the level of OXPHOS protein subunits in SAT lysates of seven discordant pairs by Western blot. Mitochondrial mass per cell (Porin, a mitochondrial outer membrane protein compared with a cytoskeletal protein beta-tubulin), trended downwards in the obese co-twins ($p = 0.09$, Figure 17 E, F). Mitochondrial OXPHOS protein subunits of CIII, CIV and CV were reduced in obesity: the level of CIII-core 2 (CIII subunit, $p = 0.018$), MT-CO1 (CIV subunit, $p = 0.018$) and CV-α (CV subunit, $p = 0.028$), compared with the levels of beta-tubulin, were all lower in the obese than in the lean co-twins (Figure 17E). As the different complex subunits typically follow the amounts of full holocomplexes, these results support deficiency of CIII, CIV, and CV. Fully nuclear-encoded CII did not differ between the co-twins. Furthermore, we analyzed whether the OXPHOS levels were reduced per mitochondria in obesity. Thus, we normalized the OXPHOS protein signals against the mitochondrial protein Porin. The amount of CI ($p = 0.063$) trended downwards, and complexes CIII ($p = 0.018$) and CIV ($p = 0.028$) were reduced in the obese co-twins (Figure 17F). Overall, the protein results suggested a trend of lower mitochondrial mass (Porin), and a decrease of OXPHOS levels per cell and per mitochondria as a downstream effect of the reduced mtDNA amount.

Figure 17: Reduction of PGC-1α, mtDNA amount, mtDNA transcripts, MRPS/MRPL and OXPHOS protein amounts in SAT of obese MZ twins.

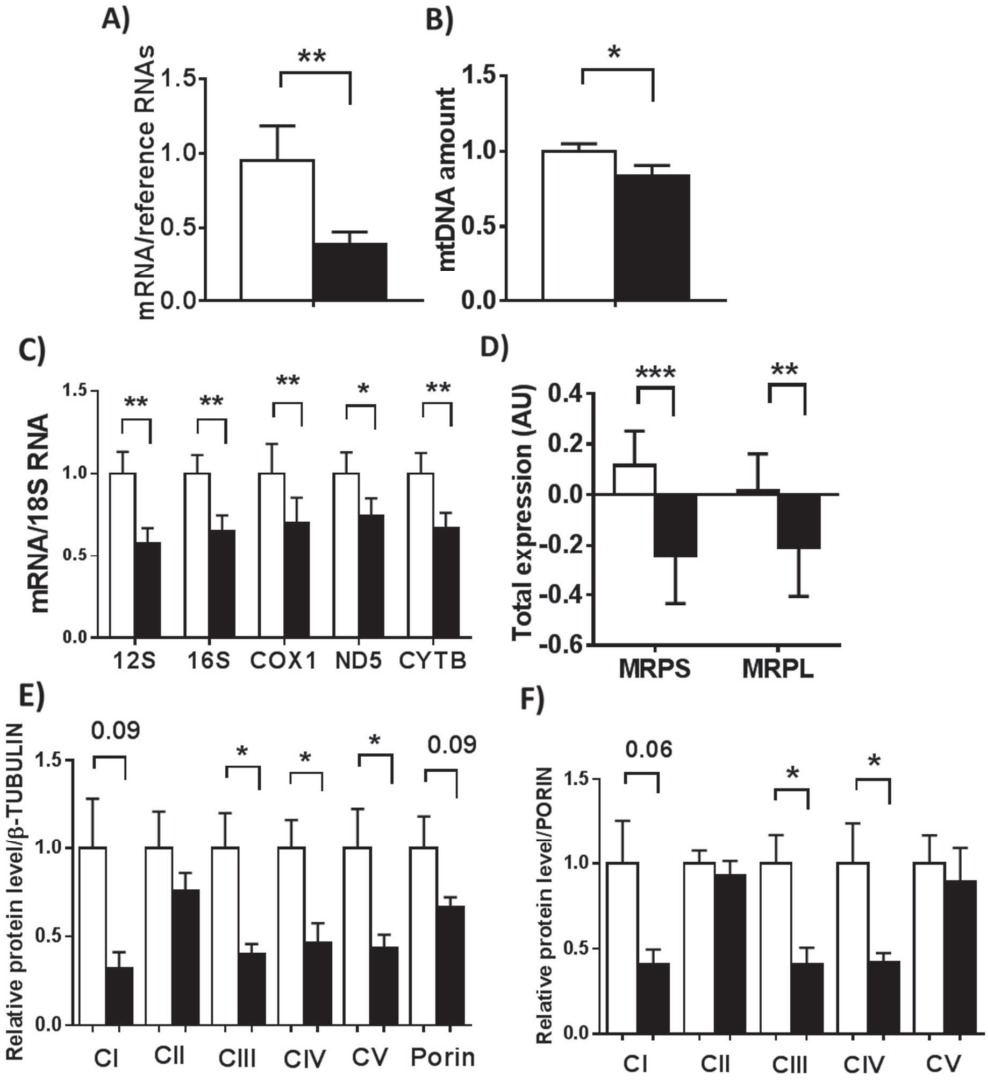


Figure 17: *A. Reduced expression of the main mitochondrial regulator PGC-1α (qRT-PCR) in SAT of the obese co-twins (n=15 discordant pairs). White bars represent lean twins and black bars obese twins. ***P<0.001, **P<0.01, *P<0.05, paired Wilcoxon signed rank test. Error bars indicate mean ± standard error. B. Relative mtDNA amount measured by qPCR and C. mtDNA transcripts measured by qRT-PCR in SAT of the MZ obese compared with the lean co-twins (n = 15 discordant pairs). D: The mean centroid values of MRPS and MRPL in the SAT of the obese compared with the lean co-twins (n = 26 discordant pairs). E and F. Relative amount of OXPHOS proteins in the SAT (n = 7 discordant pairs). Western blot signals were normalized against cytosolic β-tubulin (E) and mitochondrial membrane protein Porin (F).*

Figure 18: Representative Western Blot bands from three twin pairs

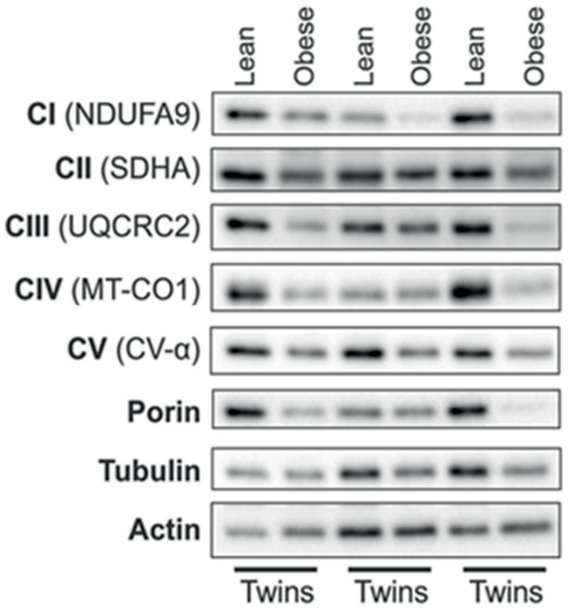


Figure 18: *Western Blot bands from three twin pairs. CI-CV: complexes 1-5. Actin, Porin and Tubulin were used as controls.*

6.5. Obese adipocytes show distinct reduced transcripts related to mitochondrial genes and proteins (IV)

6.5.1 Significantly differentially expressed genes between the co-twins in adipocytes revealed mitochondrial downregulation and an inflammatory pattern

Our previous studies on adipose tissue raised an interesting question of whether the changes in gene expression and worsening of the metabolic profile in the obese co-twins in acquired obesity were derived from adipocytes or from other cells in the adipose tissue. In addition to adipocytes, adipose tissue includes a population of stromal vascular fraction cells, including immune cells. In study IV, we wanted to investigate the transcript profiles of obese adipocytes and the similarities or differences between their abundance in adipocytes and those of adipose tissue. We started with the genes and pathways that were significantly changed between the 14 discordant co-twins. The transcriptomics analyses of the adipocytes revealed that these significantly differentially expressed genes between the co-twins were related to oxidative phosphorylation, glutaryl-CoA degradation, mTOR-signaling as well as BCAA catabolism (valine- and isoleucine degradation) and, which were all downregulated in the heavier co-twins ($p < 0.05$ all, Table 4). Glucocorticoid-receptor signaling and IL-8 signaling were upregulated in the heavier co-twins ($p < 0.05$ all, Table 4).

To see whether these downregulated pathways had any clinical implication for the obese co-twins, the top ten pathways of both adipocytes and adipose tissue were correlated with metabolic measures of the body. For IL-8 receptor signaling and glucocorticoid receptor signaling in adipocytes, the correlations were opposite to the above mentioned mitochondrial pathways. Mitochondria-related pathways of oxidative phosphorylation, valine, isoleucine degradation and glutaryl-CoA degradation correlated negatively with many adiposity and insulin resistance measure, hs-CRP and adipsin and positively with Matsuda-index and adiponectin (Table 5 A and B). For IL-8 receptor signaling and glucocorticoid receptor signaling, the correlations were opposite to the above mentioned mitochondrial pathways. JAK2 signaling, NRF2-mediated oxidative stress response and EIF4 signaling did not correlate with clinical measures (Table 5 A and B).

To detect transcripts that regulate the observed genes and pathways, we did an IPA upstream regulator analysis for the significantly differentially expressed genes. This analysis revealed *SREBF1*, *CEBPA* and *MYC* as top significant regulators. However, when compared with a t-test none of these transcripts were significantly different between the co-twins. We then combined the significantly different genes with the Mitocarta gene list to detect specifically mitochondria-related regulators. Here, *SREBF1*, *MYC* and *PGC-1α* emerged as top three regulators. PGC-1α was the only transcription factor significantly reduced in the heavier (3.9±0.3) compared with the leaner (4.3±0.3 Affymetrix units (AU), $p = 0.0157$) co-twins and this result was confirmed by qRT-PCR (heavier 2.6±0.6, leaner 4.1±1.0, $p = 0.0414$, Figure 19A).

We then did a consistency analysis, where we looked at all the genes which were both significantly different between the co-twins but also consistently up- or downregulated in the heavier co-twin. The consistency limit was set to at least 12 out of 14 twin pairs. This analysis revealed that adipocytes had 454 consistently upregulated and 538 consistently downregulated transcripts in the heavier co-twins. The downregulated genes were related to mitochondria-related pathways (oxidative phosphorylation, fatty acid β-oxidation, AMPK signaling, glutaryl-CoA degradation, TCA cycle II; $p < 0.001$ all), while the upregulated pathways displayed an inflammatory pattern (IL-10 signaling, granulocyte adhesion, IL-8 signaling, recognition of bacteria and viruses, HMGB1 signaling and cytokine production; $p < 0.001$ all).

Table 4: Top 10 pathways in adipocytes of the heavier compared with the leaner co-twins (IPA analysis of the significantly differentially expressed genes, n = 14 discordant twin pairs)

Ingenuity canonical pathways	Ttest *P*-value	Regulation
Oxidative phosphorylation	0.0019	Downregulated
Valine degradation I	0.0035	Downregulated
Glucocorticoid Receptor Signaling	0.0029	Upregulated
IL-8 Signaling	0.0012	Upregulated
mTOR Signaling	0.0043	Downregulated
Role of JAK2 in Hormone-like Cytokine Signaling	0.9750	Not changed
Isoleucine Degradation I	0.0012	Downregulated
Glutaryl-CoA Degradation	0.0012	Downregulated
NRF2-mediated Oxidative Stress Response	0.0736	Not changed

IPA, Ingenuity Pathway Analysis

Table 5A: Correlations of top 10 pathways with metabolic measures in adipocytes in individual MZ twins (n = 36 twins)

Pathway	Fat kg	SAT	VAT	Liver fat	Adipocyte volume	Insulin
Oxidative Phosphorylation	-0.51*	-0.42	-0.70***	-0.55**	-0.51	-0.49
Valine Degradation	-0.56**	-0.48*	-0.66***	-0.61***	-0.57	-0.53**
Glucocorticoid Receptor Signaling	0.48*	0.44	0.67***	0.58**	0.42	0.38
IL-8 Signaling	0.64***	0.61**	0.84***	0.64***	0.59*	0.53**
mTOR Signaling	-0.39*	-0.42**	-0.46**	-0.41*	-0.51**	-0.33
JAK2 in Hormone-like Cytokine Signaling	0.33	0.25	0.16	0.25	0.32	-0.02
Isoleucine Degradation I	-0.55**	-0.43	-0.62**	-0.62**	-0.55	-0.52**
Glutaryl-CoA Degradation	-0.62**	-0.54**	-0.72***	-0.65***	-0.66**	-0.55**
NRF2-mediated Oxidative Stress Response	0.23	0.18	0.25	0.09	0.11	0.16
EIF4 and p70S6K Signaling	-0.37	-0.38	-0.44	-0.39	-0.46	-0.34

Table 5B: Correlations of top 10 pathways with metabolic measures in adipocytes in individual MZ twins (n = 36 twins)

Pathway	HOMA	Matsuda	Leptin	Adipo nectin	hs-CRP	Adipsin
Oxidative Phosphorylation	-0.51	0.61**	-0.24	0.48***	-0.41	0.19
Valine Degradation	-0.54**	0.68***	-0.38	0.50*	-0.46*	0.04
Glucocorticoid Receptor Signaling	0.37	-0.61**	0.31	-0.49**	0.49*	0.38
IL-8 Signaling	0.54**	-0.70***	0.40*	-0.44**	0.57**	0.07
mTOR Signaling	-0.32	0.39*	-0.31**	0.14	-0.28	0.26
JAK2 in Hormone-like Cytokine Signaling	-0.01	-0.12	0.24	-0.04	0.26	-0.43*
Isoleucine Degradation I	-0.52**	0.65***	-0.35	0.56**	-0.46	-0.50*
Glutaryl-CoA Degradation	-0.55**	0.70***	-0.44*	0.50**	-0.47	-0.45
NRF2-mediated Oxidative Stress Response	0.16	-0.20	0.13	-0.43**	0.09	-0.26
EIF4 and p70S6K Signaling	-0.36	0.35	-0.20	0.07	-0.18	0.08

6.5.2 Reduction of mtDNA transcripts, *PGC-1α* (qRT-PCR) and mitochondrial ribosomal protein subunits (Affymetrix units) in adipocytes of the heavier co-twins

To further investigate specifically the transcripts of mitochondrial genes that are not detectable with Affymetrix array, we did a qRT-PCR of mtDNA-encoded rRNAs 12S, 16S RNA and mRNAs *COX1, ND5* and *CYTB*. Mitochondrial 12S (*MT-RNR1*, $p = 0.0022$) and 16S (*MT-RNR2*, $p = 0.0186$) rRNAs, as well as mRNAs *COX1* ($p = 0.0047$, CIV subunit), *ND5* ($p = 0.0186$, CI subunit), and *CYTB* ($p = 0.0047$, CIII subunit) were all significantly downregulated in adipocytes of the heavier as compared with the leaner co-twins (Figure 19B).

The mitochondrial mRNAs are translated on mitochondrial ribosomes into proteins which form parts of the OXPHOS complexes. To shed light on the level of the nuclear transcripts encoding subunits of these ribosomes we calculated mean centroids (an average mean) for the transcripts of small (MRPS) and large (MRPL) mitochondrial ribosomal protein subunits in adipocytes for n=29 (MRPS) and n=49 (MRPL) Affymetrix transcripts. This analysis showed a downregulation of mitochondrial ribosomal protein subunits in the heavier co-twins (MRPS: heavier -0.24 ± 0.18 vs. leaner 0.23 ± 0.18 AU, arbitrary Affymetrix units, $p = 0.0092$; MRPL: heavier -0.17 ± 0.18 vs. leaner 0.18 ± 0.17 AU, $p = 0.0157$, Figure 19C).

In upstream regulator analyses, when combining the significantly differentially expressed genes with Mitocarta gene list of mitochondria-related transcripts, the top regulators detected were *SREBF1*, *MYC* and *PGC-1α*. *PGC-1α* being one of the master regulators of mitochondrial biogenesis and found to be the only transcription factor significantly reduced in the heavier (3.9±0.3) compared with leaner (4.3±0.3 Affymetrix units (AU), $p = 0.0157$) co-twins, we then verified the result in a

qRT-PCR of *PGC-1α* in adipocytes with the heavier co-twin having reduced levels of this transcript (heavier 2.6± 0.6, lean 4.1±1.0, $p = 0.0414$, Figure 19A).

6.5.3. Reduction of OXPHOS protein amounts in obese versus lean individuals

In seven lean and six obese individuals the Western Blot analysis of mitochondrial OXPHOS protein subunit levels of CI, CII, CIII, CIV and CV were studied: the levels of CI subunit NDUFB8 ($p = 0.0176$) and CIII-core 2 subunit (CIII, $p = 0.0455$) were reduced in the obese compared with the lean individuals and COX2 (CIV subunit, $p = 0.253$) and ATP5A (CV subunit, $p = 0.254$), were lower but non-significant in the obese compared with the lean individuals (Figure 19D). The nuclear-encoded CII subunit SDHB did not differ between the obese and the lean ($p = 0.361$). When the OXPHOS protein signals were normalized against the mitochondrial protein porin, complex CI subunit NDUFB8 ($p = 0.0106$) was significantly reduced in the obese compared with the leaner individuals, suggesting a decrease of OXPHOS CI levels per mitochondria. CIV ($p = 0.086$) trended downwards and CII ($p = 0.988$), CIII ($p = 0.199$), and CV ($p = 0.775$) were non-significant in the obese compared with the lean individuals (Figure 19E).

Figure 19: Reduction of PGC-1α, mtDNA transcripts and MRPS/MRPL expression in adipocytes of the heavier MZ twins. Reduction of OXPHOS protein amounts in obese individuals.

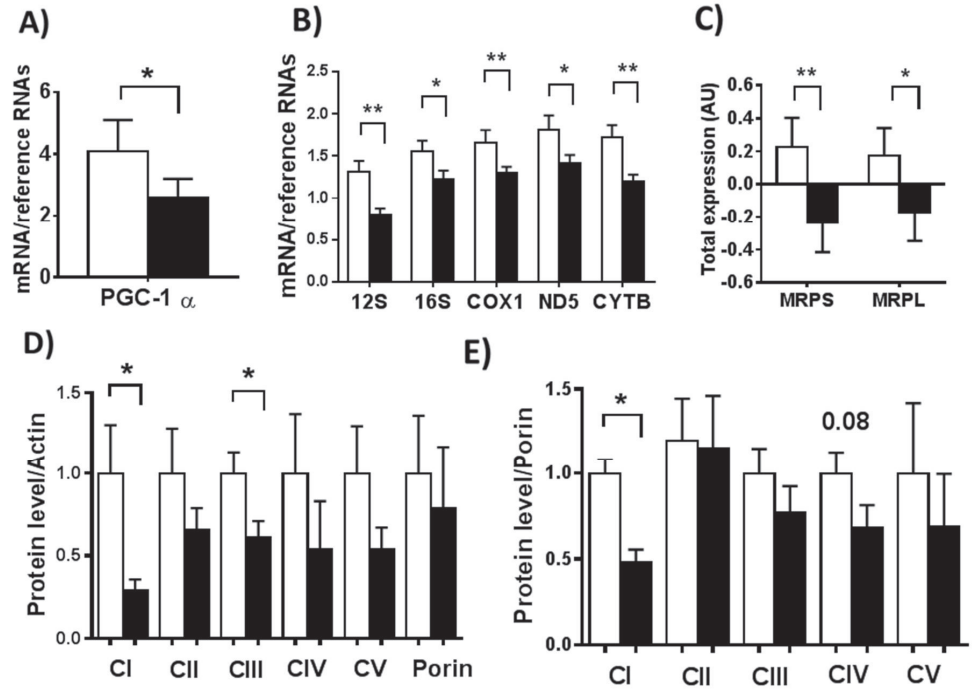


Figure 19: *A) Reduced expression of the main mitochondrial regulator PGC-1α (qRT-PCR) in adipocytes of the heavier co-twins (n=12 discordant pairs). White bars represent lean twins and black bars heavier twins in the figures A-C. ***P<0.001, **P<0.01, *P<0.05, paired Wilcoxon signed rank test. Error bars indicate mean ± standard error. B) mtDNA transcripts measured by qRT-PCR in adipocytes of the MZ heavier compared with the lean co-twins (n = 12 discordant pairs). C) The mean centroid values of mitochondrial ribosomal protein small (MRPS) and large (MRPL) subunits in the SAT of the obese compared with the lean co-twins (n = 15 discordant pairs). D and E) Relative amount of OXPHOS proteins in adipocytes (n = 4 obese and n=4 lean individuals). Western blot signals were normalized against cytosolic Actin (D) and mitochondrial membrane protein Porin (E).*

Figure 20: Western Blot bands from two pairs of lean and obese individuals

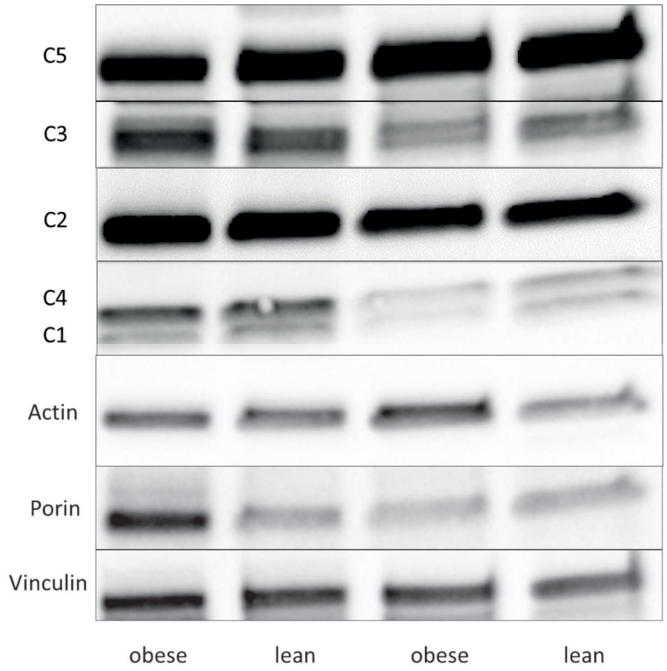


Figure 20: *Western Blot bands from two lean and two obese individuals. C5: complex V, C3: complex III, C2: complex II, C4: complex IV, C1: complex I. Actin, Porin and Vinculin were used as control proteins.*

6.5.4. Adipose tissue transcriptomics in 14 discordant twins mimicked those of the adipocytes

The same transcriptomic analyses of the 14 discordant co-twins in adipose tissue revealed a total of 2135 transcripts that were differentially expressed between the co-twins (nominal *P* value <0.05). As in adipocytes, the top 10 upregulated genes in the heavier co-twins (Study IV, Supplementary Data in figshare repository, https://dx.doi.org/10.6084/m9.figshare.3806286.v1) were associated with immune reactions and cellular stress and the top 10 downregulated transcripts (Study

IV,supplementary data in figshare repository) with mitochondria, fatty acid synthesis and β-oxidation. Analyses according to the fold change between the co-twins revealed chemokines and inflammatory genes among the top upregulated list as in adipocytes. Also, the pathway-analyses in adipose tissue mimicked those of adipocytes, with oxidative phosphorylation, valine and lysine degradation, glutaryl-CoA degradation, acetate conversion to Acetyl-CoA, fatty-acid β-oxidation, triacylglycerol synthesis and ketone body production and breakdown among top 10 pathways, all downregulated in the heavy co-twins ($p < 0.05$ all). Strong negative correlations were found for the top 10 pathways and adiposity, including adipocyte size, leptin, insulin resistance, hs-CRP and adipsin and positive correlations with Matsuda-index and adiponectin. Triacylglycerol synthesis was an exception correlating only with intra-abdominal and liver fat and adipsin. The mitochondrial top three upstream regulators in adipose tissue were *SREBF1*, *PGC-1α* and *EIF2A* of which *PGC-1α* (3.8±0.3 vs. 4.9±0.3 AU, $p = 0.0019$) and *EIF2A* (11.2±0.04 vs. 11.4±0.1 AU, $p = 0.0029$) transcript levels were reduced in the heavier co-twins.

In consistency-analyses the top significantly downregulated pathways included CHK proteins in cell cycle control, DNA modifications, cysteine degradation, mTOR signaling, cholesterol biosynthesis, dopamine signaling, and upregulated pathways 14-3-3-signaling, NF-κB activation, immune cell cytokine communication, lipid antigen presentation, CD40-signaling, androgen signaling and GABA receptor signaling ($p < 0.001$ all).

When repeating the transcriptomics analyses on mitochondrial ribosomal protein subunits, both MRPS (-0.24±0.19 vs. 0.11±0.14 AU, $p < 0.01$) and MRPL (-0.21±0.2 vs. 0.01±0.15 AU, $p < 0.05$) were downregulated in the heavier co-twins as in adipocytes.

6.6. Sex, onset of obesity-discordance, family history and lifestyle factors (diet, alcohol intake, smoking and physical activity) in the discordant twin pairs (I-IV)

Although the differences between sexes in body composition and fat distribution are well known, there were no within-pair differences in the measures of adiposity and metabolism or in the measures of mitochondrial activity in males and females. The onset of obesity-discordance in all 26 discordant twin pairs was 22.6 ± 0.6 years (437) in the early adulthood of the twins, and the length of the discordance on average 6.3 ± 0.3 years. Self-reported reasons for the weight gain for the heavier co-twins were behavioral: eating unhealthier and exercising less than their leaner counterparts (438). In the family history of the twins, of the total 44 parents of the discordant pairs, for whom data was available, 15.9% were obese (4/23 of the mothers, mean age 42.2 ± 0.7 years and 3/21 of the fathers, mean age 45.4 ± 0.8 years), including one twin pair where both parents were obese. Physical activity of the twins was measured by Baecke-index and its three sub-components (sport, leisure time and work indexes) from the twin questionnaires. There were no significant differences in total- as well as sport- or leisure-time physical activity indexes between the co-twins. In study II, the metabolically different groups 1 and 2 were similar with regards to age, sex, smoking habits, alcohol intake, total physical activity and mean daily energy intake. In group 2, sports activity was lower and alcohol intake tended to be higher in the obese than in the lean co-twins.

7. DISCUSSION

7.1. Summary of the main findings

This thesis shows that mitochondrial biogenesis is reduced in obese adipose tissue and adipocytes, both at the transcript level as well as at the protein level. This reduced biogenesis is associated with the metabolic abnormalities of obesity. Adipocyte hypertrophy with little hyperplasia is related to worsening of the metabolic profile in obesity. The hyperplastic capacity of adipose tissue in an individual may be genetically determined. We show in twins that there are two metabolically distinct subgroups of obesity, which can be separated according to the amount of liver fat relative to their leaner co-twin. The "metabolically unhealthy" group had increased liver fat amount compared to their leaner co-twin. This group also presented with low capacity for adipocyte hyperplasia in their adipose tissue. The two groups had the most distinct differences in mitochondria- and inflammation-related genetic pathways. Mitochondria-related transcriptomics pathways as well as mitochondrial DNA transcripts and mitochondrial OXPHOS protein subunits were downregulated in the obese co-twins, suggesting a general reduction of mitochondrial biogenesis in obesity. The mitochondrial transcriptomics pathways also correlated negatively with the metabolic measures of obesity, suggesting that their reduced activity is related to a worsening of the metabolic profile. In our results the downregulation of the essential functions of mitochondria in adipose tissue in obesity was not only a phenomenon of adipose tissue, but strongly influenced by the adipocyte cells.

A general assumption is that at a certain level of adipocyte hypertrophy, a critical threshold emerges, at which the cells cannot ingest more fat and begin exhibiting signs of stress. At this point the lipid flux to ectopic tissues increases causing toxic effects like insulin resistance and apoptosis. Low mitochondrial function, adipocyte membrane stress, adipose tissue hypoxia, increased influx of FFAs, altered adipokines and increased inflammatory cytokines are some of the factors suggested to lie behind the adipocyte stress reaction. The correlation between fat mass and metabolic problems however is not linear. The reasons for this have thus far remained unknown. In this thesis, we suggest that increased adipocyte size but low capacity to hyperplasia is a factor directly related to metabolic abnormalities in adipose tissue and in the whole body and also discerning individuals who are prone to the metabolic complications of obesity. In addition to high hyperplastic capacity and small adipocyte size, "metabolically healthy" obesity was distinguished by low liver fat content in the obese twins and a decrease in mitochondrial activity by fatty-acid and BCAA oxidation and absence of inflammation in their adipose tissue. These results backed up the hypothesis that if subcutaneous adipose tissue expandability is good, metabolic problems are better prevented. Finally, we linked the metabolic dysfunction in obesity to low mitochondrial amount and biogenesis in adipose tissue. We also suggest that at the transcript level, the downregulation of mitochondrial pathways is connected especially to the metabolically unhealthy obesity, but not to the healthy phenotype. The same changes of acquired obesity were seen both in adipose tissue and adipocyte cells.

7.2. Adipocyte hypertrophy, hyperplasia and metabolic health in acquired obesity

In this thesis, we have associated adipocyte volume to a worsening of metabolic profile in obesity in study I (430). Based on the hypertrophy of the adipocytes and the hyperplastic capacity of adipose tissue in the obese twins, the twins could be divided into two metabolically different groups (430). In study II, the twins who were characterized as metabolically unhealthy with increased liver fat content compared with their leaner co-twins and worsened metabolic measures compared with the metabolically healthy obese in group 1, also had hypertrophied adipocytes and less hyperplasia in their subcutaneous adipose tissue. We have also presented negative correlations between mitochondrial oxidative pathways and adipocyte volume in study III (437) in twins. These findings support the idea of adipocyte volume and adipocyte morphology (as measured by adipocyte volume/hypertrophy and adipocyte number/hyperplasia in an individual) as an important factor in obesity-related complications. Also, when correcting against kg body fat, negative correlations between adipocyte volume and metabolic measures in obese twins remained, suggesting an independent effect of total body fat in metabolic complications.

In study I we noticed that although adipocyte volume increased with increasing obesity, adipocyte number between the co-twins remained same, both in MZ discordant and concordant co-twins. Our results suggest that subcutaneous adipocyte morphology is under genetic control, as evidenced by the remarkably similar adipocyte cell number and size (ICC 0.91-0.92) in weight-concordant MZ co-twins. In weight-discordant pairs, the responses to obesity varied: some obese co-twins reacting through hypertrophy and others through hyperplasia combined with a milder hypertrophy in their adipocytes. In the hypertrophic obesity group, the obese co-twins had a lower adipocyte count, higher liver fat, and disturbances in their insulin sensitivity as well as several other metabolic parameters compared with their lean co-twins. In contrast, hyperplastic obese co-twins in the latter group were as healthy as their lean twin pair members. The increase in adipocyte cell size within the twin pairs was associated with decreased expression of mitochondrial genes as well as with increased expression of genes regulating cell death and inflammation.

Obesity is known to be highly heritable. Study I of the thesis suggests that this may also hold true for the adipocyte size and number. Consistent with our results, the seminal overfeeding studies in MZ twins have showed that the members of weight-concordant pairs resembled each other significantly for fat cell size and fat cell lipolytic activities (439). In the present study the obesity-discordant MZ pairs were less similar for fat cell size (intra-class correlation ICC=0.34) than for fat cell number (ICC=0.63), which suggests that acquired obesity significantly influences adipocyte volume, whereas the control of cell count could be more genetic. This is in line with studies in rats where adipocyte size was increased by high-fat feeding independent of genetic factors, whereas increase in adipocyte number was enhanced by high-fat diet only in certain strains (440). Several genetic causes of human lipodystrophies have been discovered in the past years, also proposing that the presence of genetic factors underlie adipogenic capacity (5).

Spalding et al 2008 reported that neither adipocyte death rate nor adipocyte generation rate were altered in obese compared with lean unrelated individuals, speaking for a tight regulation of adipocyte number in adulthood, even in the obese state (120). Although absolute fat cell production

was higher in obese than in the lean individuals, the rate of adipocyte generation was same in both groups. Because the fat mass in the lean and in the obese both stayed at the same level, this suggests that the difference in the number of adipocytes in obese versus lean individuals occurs already before adulthood (120). Hyperplasia however seems to correlate with the severity of obesity. Morbidly obese individuals show more evident hyperplasia than obese or lean individuals (441). In obese (442) and type 2 diabetic subjects (443), a lower differentiation potential and reduced generation of preadipocytes has been proposed, but individual differences in the differentiation capacity of preadipocytes in obesity had not been assessed before. The similarity of MZ concordant co-twins in our study suggested a strong genetic component in the regulation of adipocyte number in adulthood. In the hypertrophic obesity group of our study, even as the discordant twins acquired more fat, their total adipocyte number stayed in a comparative level or even decreased compared with their leaner co-twins, but in the hyperplastic group, the obese co-twins had acquired more cells than their leaner counterparts. Our study thus proposes that some individuals are able to respond to long-term energy excess with hyperplasia, while others predominantly react by hypertrophy. This difference could be a factor behind different metabolic outcomes of obesity. Acquired obesity in the present study was characterized in particular by a reduction in small adipocytes. Although it is generally regarded that small adipocytes are more insulin sensitive, data on their decreased amount or causal role in the development of metabolic derangements in obesity is controversial (227).

Regarding the metabolically healthy-obesity phenotype, smaller adipocytes have been reported in omental adipose tissue of morbidly obese but insulin sensitive individuals (14) as well as in individuals characterized as “metabolically healthy” obese (224), without insulin resistance or any other features of metabolic syndrome (410). Adipocyte size distribution in these studies was measured in visceral and subcutaneous abdominal surgery samples (14, 410). McLaughlin et al. have reported larger mean adipocyte size in subcutaneous adipose tissue of both overweight and obese insulin-sensitive and insulin-resistant humans, challenging the idea of the relationship between adipocyte volume and insulin resistance. Contrary to the expectations, the small-to-large adipocyte ratio was higher in the insulin-resistance group, suggesting that the insulin resistant obese had more small cells than the insulin sensitive obese individuals (227). The study also presented with 2–3-fold increase in the expression of genes encoding markers of adipocyte differentiation. Together, the findings could suggest an impaired adipocyte differentiation by the arrest of development in the very small adipocytes toward fully mature cells in insulin resistant obese individuals, explaining the larger ratio of small cells in insulin resistant individuals (227). It could be thus postulated that the insulin sensitive obese individuals who have better adipocyte differentiation from very small cells to mature ones and thus adipose tissue storage capacity, would be protected from the adverse effects of ectopic fat accumulation. However, in study I of this thesis, based on the extreme similarity of MZ twins in their total adipocyte cell count even in the obese state, the adipocyte differentiation capacity seems to be determined by genes. Thus, the obese who develop insulin resistance may not have enough preadipocytes for differentiation to start with, or the differentiation capacity of the cells may be lower than in the more healthy subjects, and these factors may be determined by genes. Our study did not assess the large and small fat cell pools in relation to insulin sensitivity or mitochondrial function, but a significant general correlation was observed between these variables. In article I, increased adipocyte volume in the obese twins was associated with an impaired mitochondrial function and downregulation of gene expression pathways in lipid handling and

steroid metabolism, structural changes in the cell membranes, DNA damage as well as higher cell death and inflammation. These features associated with the development of metabolic disturbances in obesity. Our hypothesis, based on the results of the pathway-analyses in study I of this thesis and backed up by the other three studies is that downregulation of mitochondrial biogenesis is associated with disturbances in adipocyte lipid handling and fatty acid oxidation. Increased lipid load by obesity and the decreased fatty acid oxidation in mitochondria lead to the accumulation of unoxidized FFA and intermediary metabolites like monomethyl BCAAs in adipocytes (444). Disruptions in the adipocyte mitochondrial function have indeed been shown to lead to enhanced lipid deposition in the cell (445) in such amounts that the ROS production was increased, leading to ER stress and increased FFA spillover to ectopic sites (65, 243, 256). In line with these studies our results suggest that the stressed mitochondria release more ROS, which leads to adipocyte stress and the expression of inflammatory genes and the secretion of inflammatory cytokines. Apart from nuclear-related changes in the adipocyte membrane plasticity, mitochondria control the synthesis of the phospholipids of the cell surface (153), a process which can be influenced by low mitochondrial function. The cell membrane changes result in the recruitment of macrophages into adipose tissue (19) and finally to the crown-like inflammatory structures around extra-large cells, adipose tissue cell death and enhanced inflammation. Inflammatory cytokines in turn can harm mitochondria, decrease mitochondrial biogenesis and the function of the OXPHOS system. Thus, once initiated, the changes seen in obese dysfunctional adipose tissue may be a vicious circle.

Indeed, already previously a study from our group has associated obesity with membrane lipid alterations in adipocytes and suggested that these alterations trigger the recruitment of inflammatory cells to the adipose tissue (19). We have also shown that inflammatory pathways were upregulated and mitochondrial BCAA-catabolism, cell differentiation and energy metabolism pathways downregulated in obesity in twins (262). These findings are also in line with a previous study of an inflammatory profile in large adipocytes (231). Certain individual interesting genes also emerged. We (430), and previously others (231) observed that adipocyte hypertrophy was linked to the increased expression of palladin (PALLD), which is a cytoskeletal protein working in adhesion and migration of the cells. Another gene, the function of which was related to cell-to cell interactions, membrane modifications and actin cytoskeleton organization (446), was moesin (MSN). Indeed, it has been proposed that in obesity, there is an active reorganization of the adipose tissue structure, specifically visible in its gene expression patterns (352). Moesin was also important for apoptosis (447) and oxidative stress (448). We also found PUMA (BBC3), which induces mitochondrial membrane permeabilization and thus apoptosis in the cell (449). Inflammatory genes were well presented with MIF, PKAR2B, RNF125 and PLA2G4A correlating strongest with the adipocyte volume. We also observed downregulation of CIDEA, an important mediator of lipolysis (450) and triglyceride deposition in the adipocytes (451). The downregulation of CIDEA in adipose tissue has been suggested to contribute to the ectopic lipid accumulation (451).

In study III (437), the main finding was that adipocyte hypertrophy was negatively correlated to the expression of various oxidative and metabolic-related pathways in mitochondria, speaking for an important assocciation of adipocyte size to mitochondrial function in the cell. Our study on reduced expression of oxidative pathways and reduced levels of the subunits of the OXPHOS system verified a general downregulation of OXPHOS subunits in obesity, correlating with general adipocyte size.

However, we did not assess the reduction of mitochondria-related pathways and OXPHOS protein levels separately in the small or large adipocytes of the tissue. A study of Yin et al 2014 found no differences between the obese and lean individuals in the respiratory capacities of small and large adipocytes (10). Fisher et al 2015 suggested that small and large adipocytes would not be different in their oxidative capacity per se, but generally all adipocytes from an obese or a lean individual would have the same oxidative profile, the metabolic profile affected by obesity itself (235). This would suggest that adipocyte volume as a single measure might not be an independent marker of the oxidative status of the cell. Based on the controversial findings, the relationship of adipocyte hypertrophy and mitochondrial respiratory activity will probably need more studies.

As adipocyte size has been linked to adipocyte cell death (208) rather than overall obesity, and suggested to be the main factor contributing to the inflammatory macrophage infiltration in adipose tissue in mice (208), it could be plausible that adipocyte volume is a marker of the dysfunction of the tissue, at least in mice. In humans, adipocyte hypertrophy has been linked to the increased secretion of inflammatory proteins, enhanced lipolysis in adipose tissue and decreased insulin-induced glucose uptake (452), all factors that are related to the development of insulin resistance. Transcriptomic profiles of separated adipocytes demonstrated that large adipocytes express higher levels of inflammatory genes than small adipocytes (231). Adipocyte volume as a measure is however always linked to the accumulation of total body fat and is also affected by sex (223). Adipogenic capacity is affected by sex and adipose tissue depot. Adipose precursor cells have distinct patterns of gene expression, differentiation potential, and response to environmental effects depending on their depot and the sex of the individual (220). Arner et al showed that in the obese range of the adipocyte size, adipocyte volume may also be a biased measure. The relationship between adipocyte size and body fat mass is curvilinear and reaches a plateau at certain fat cell sizes in both men and women (453). When adipocyte volume reaches its maximal size, the excess fat starts to accumulate to ectopic sites. Near the maximal volume of the cell the adipocyte size does not anymore increase linearly, but approaches a plateau, and the volume thus no longer presents the real metabolic state of the tissue (441). There may also be differences between men and women in reaching this adipocyte hypertrophy plateau and thus difference in adipocyte hyperplastic capacity, as suggested in the meta-analysis by Laforest et al 2015. In their study, it was noted that men are more prone to hypertrophy of adipocytes, reaching the adipocyte size plateau at lower BMI values ($25 kg/m^2$) than women ($35 kg/m^2$). Also omental adipocyte size has been shown to be 20% smaller than subcutaneous adipocyte size in women, while in men there is no difference in these measures (454). Arner et al were also able to show, that subjects with larger adipocyte volume than predicted by the curve of increasing adipocyte hypertrophy, had lower rates of adipogenesis and that adipogenesis correlated negatively to adipocyte hypertrophy, when adjusted with the predicted volume at a given body fat mass (453). Despite all this, it has still been unclear, to which extent an individual can acquire additional fat cells in adulthood. Most studies done related to adipocyte hypertrophy and hyperplasia are cross-sectional, and in short-term weight increase, no significant increase in adipocyte number has been observed (455). However, it seems that fat cell number at least does not decrease in adulthood, even following long-term weight loss (120).

While definitive cause and consequence- determinations have not been made, a general assumption is that problems in adipose tissue in obesity precede the metabolic problems in other organs and whole body. It is interesting however, that the relationship of adipocyte size and the development of the metabolic problems of obesity doesn't seem to be linear either. Different individuals develop

metabolic complications of obesity at different adipocyte hypertrophy levels. Taking into account both adipocyte volume and hyperplasia – a morphology value, suggested by Arner et al (453) ─ could indeed be a better predictor of metabolic problems. It has been suggested that individuals with adipocyte hypertrophy are less capable of storing fat in their adipocytes than individuals with capacity to adipocyte hyperplasia (362) and that the reduced adipose tissue storing capacity in hypertrophic, but not in hyperplastic obesity would enhance ectopic lipid accumulation and related problems (362). In humans, adipose tissue cellularity has been connected to the inflammatory and metabolic status of adipose tissue (46). Metabolically normal obese individuals have been shown to have elevated levels of proadipogenic factors such as Wnt signaling regulator Dapper (456) and increased lipid droplet protein levels (Perilipin, Cidea, FSP27), suggesting an improved adipose tissue hyperplastic capacity and function compared to metabolically compromised individuals. In a recent study, adipocyte morphology predicted the outcomes of improvement in insulin sensitivity in weight loss, distinguishing the individuals that had the largest improvements in their insulin sensitivity – those with the hypertrophic but not hyperplastic obesity phenotype (457). Interestingly, adipocyte morphology in this study was not associated with the amount of weight loss but rather to its outcomes. The calculation of morphology might be important in determining, which patients are at highest risk of problems related to obesity and would best benefit from weight reduction therapies (457).

In study I of this thesis, we showed that based on adipocyte size and number in whole body, twins could be divided into hyperplastic and hypertrophic groups - a form of morphology value – where hypertrophic twins clearly had a worsened metabolic profile compared to the hyperplastic group. Early detection of individuals that are most prone to obesity-related complications would be a great health benefit. No differences in adipocyte number between the obese and the lean MZ concordant co-twins, but an increase in the number of cells in the obese co-twins of the hyperplastic group, suggested that the adipocyte number and thus possibly also the hyperplastic capacity is genetically determined. This raises questions on an early detection of different adipose tissue cellularity phenotypes in obesity. However, if the genetic background predisposes an individual to a certain adipose tissue hypertrophic and hyperplastic capacity, the increase in adipocyte volume and the metabolic complications that arise after excessive fat accumulation seem to be caused by acquired obesity, confirmed by comparing the obese and the lean co-twins in our study.

It is interesting, however, that while many studies have used the mean fat cell diameter in their analyses, this approach may miss the very small and the very large adipocytes and the subpopulations of the cells, because by conventional collagenase digestion and imaging methods, very small adipocytes may not be recognized and large adipocytes may break before analysis. Cell flux analyzers and an osmium staining are some alternatives for this. An interesting question for future analyses is, if adipose tissue expansion leads to different subpopulations or different small-to large cell ratios and if these factors have an effect on the metabolic profile of adipose tissue.

7.3. Liver fat content as determinant of metabolic health in acquired obesity

In study II we showed that liver fat content and adipose tissue hyperplastic capacity may distinguish the obese individuals prone to the metabolic complications of obesity. The twin-setting gave the study the possibility of discerning acquired influences behind the metabolically healthy obesity (MHO) –phenomenon. In this study, it became evident that the twins could be divided into two metabolically different groups according to the amount of their liver fat. In group 2, in which the obese co-twins had significantly elevated liver fat content compared with their leaner counterparts, the obese twins exhibited a typical response to obesity - marked insulin resistance, dyslipidaemia, disturbed responses to OGTT, higher blood pressure, downregulated mitochondrial and upregulated inflammatory transcript pathways in SAT compared with the obese twins of the healthier group 1. Adipocyte size increased in both groups, but more in the metabolically unhealthy group 2. The groups 1 and 2 did not differ for overall fatness. What was remarkable in the study was that all the clinical values in twins were still within the normal range. This suggests that the changes seen in metabolically unhealthy obesity occur already early, before clinically observed complications, and that increase in the liver fat content is a central marker in this aspect.

Already some years previously it was noticed that not all obese individuals develop the metabolic and inflammatory changes normally seen in obesity. It was thus suggested that the fat distribution in body composition and the individual function of adipose tissue might be more important determinants of metabolic well-being than body fat mass (2, 14, 16). Earlier studies had established a relationship with excess liver fat and worsening of the metabolic profile (16, 397), especially the increase in insulin resistance (397, 458). Fatty liver had also been associated with the features of metabolic syndrome independent of obesity (459) and suggested to explain why some but not all obese individuals were prone to insulin resistance and the metabolic complications of obesity – and also why even some lean individuals develop these problems (382). Fittingly, in our present study, particularly the AUC during OGTT in the obese twins in the "metabolically unhealthy" group 2 was affected. Also, the obese twins in the group 2 had significantly more intra-abdominal fat, liver fat and larger adipocyte volume, as well as increased circulating hs-CRP levels and decreased adiponectin levels. On gene expression level an increase in chronic inflammatory response pathway, decrease in adipogenesis and decrease in mitochondrial oxidative functions were recorded. We had previously associated increased inflammation and decreased expression of mitochondrial pathways to acquired obesity (262, 460), but in this new study, we show that these changes are especially related to the obese with increased liver fat in group 2. The increased response of insulin secretion, other worsened metabolic parameters, as well as the transcriptional changes already at the early stages of obesity in young otherwise healthy twins, indicated that the metabolic problems caused by acquired obesity are detectable already much before the clinically significant type 2 DM or metabolic disease criteria are filled.

Overfeeding with a high-fat high-glucose diet increased weight by 9%, but liver fat content with 2-3 fold in normal healthy subjects (380).Weight loss in turn decreases liver fat amount (381). Study II of this thesis however demonstrated, with MZ obesity-discordant twins, that the accumulation of liver fat varies among obese individuals, independent of genetic effects. Indeed, it is interesting that an increase in the amount of liver fat (382), as well as the increase in adipocyte size (441) is not

linearly related to increases in body fat mass. Furthermore, in lipodystrophies individuals with little subcutaneous fat exhibit fatty liver (461). These notions back up the hypothesis, that compromised expandability of subcutaneous adipose tissue leads to visceral, liver and ectopic fat accumulation and to metabolic problems (17, 18, 462). Our study suggests that decreased mitochondrial fat and amino acid oxidation in SAT are associated with increased liver fat accumulation and that these associations are caused by acquired obesity. Also in study II, the adipocyte differentiation pathway was downregulated and adipocyte number decreased in the obese co-twins of group 2. As mitochondria are vital in adipocyte differentiation (463), these two phenomena may be linked. Thus, we suggest that the reduction in mitochondrial biogenesis in addition to the low level of the remodeling capacity of SAT enhance liver fat accumulation. Accumulating liver fat distinguishes between metabolic health and disease in obesity. Moreover, adipocyte size may play a role in this. 21% of known variation in liver fat could be explained by adipocyte size alone and increased adipocyte size was associated with the liver fat accumulation independent of other causes (464). The interesting results in study I of the thesis, where the metabolically unhealthier twins with disturbed metabolic parameters and increased liver fat content were present in the group that had increased adipocyte volume but only little hyperplasia in their tissue, also backs up the hypothesis that the adipose tissue expandability and liver fat amount are central markers for the metabolic well-being in obesity.

Studies have shown that the levels of Acetyl-CoA and ATP, the main substrates for lipolysis (157) and de novo lipogenesis (252) in adipocytes, are reduced if mitochondrial metabolism is compromised. In our study, the subjects were measured after an overnight fast, in a state with preferential lipolysis. As expected, we did not observe differences in the transcripts of the triacylglycerol synthesis pathway. The situation however might be different postprandially. It has also been demonstrated that increased capacity in adipose tissue for lipogenesis might protect the metabolically unhealthy obese from the problems related to weight gain (465), also speaking for the importance of adipose tissue remodeling capacity in obesity.

Generally it has been thought that at least increased visceral fat is metabolically harmful for the body, and some studies have shown that insulin sensitive obese individuals have only little visceral fat (12, 350). In contrast, a study by Magkos, Fabbrini et al reported that increased total adiposity without increase in liver fat is not associated with metabolic problems of obesity (466) and also that intrahepatic fat, but not visceral fat, is linked to the metabolic abnormalities of obesity (396). This notion was supported by another study from the same laboratory, where surgical removal of omental fat did not improve insulin sensitivity in obese adults (351). However, in another study both intra-abdominal and liver fat independently of each other explained variation in serum TG, HDL cholesterol, insulin concentrations and hepatic insulin sensitivity, supporting the idea that both fat depots are important predictors for the components of the metabolic syndrome. (467). It may be possible that both visceral and liver fat indeed do contribute to the metabolic dysfunction state in obesity, and this needs more studies.

In most, but not all obese subjects, obesity is associated with marked changes in the secretory profile of adipose tissue and its macrophages with a resulting low-grade inflammatory state (299). Samocha-Bonet et al presented in a review that one hallmark of the metabolically healthy obesity is a favourable plasma inflammatory profile compared with the metabolically unhealthy individuals (408). In our study, inflammatory pathways in SAT were upregulated and also circulatory

inflammation markers elevated in the obese twins of the unhealthier group 2 compared with the obese in the healthy group 1. On the one hand, macrophage infiltration in visceral adipose tissue has been found to be significantly increased in insulin-resistant versus insulin-sensitive obese individuals, with no differences in SAT macrophage amounts (14). In another study on the other hand, obese women with high liver fat content had increased levels of inflammatory transcription factors in their SAT and higher circulating plasma lipid levels than their obese peers without liver fat (399). An intriguing fact is that also subjects with rapid weight loss after bariatric surgery (468) can have inflammatory monocytes in their circulation. This would suggest that rapid changes in either direction in body fat content may support an inflammatory response. It is still not known if individuals with rapid onset of obesity are more prone to inflammation in their adipose tissue.

Our hypothesis was that low mitochondrial biogenesis and function precedes complications like increased cell death and inflammation in obese subcutaneous adipose tissue. The low adipogenic- and expandability capacity of the tissue may be genetic, but the causality between this and the low mitochondrial function is not yet known. However, both decreased mitochondrial function and low hyperplastic capacity of SAT are thought to lead to liver fat accumulation and to a worsened metabolic profile in obesity. The process may however be a vicious circle. A study by Vernochet et al showed that inducing decreased expression and enzymatic activity of complex I, III, and IV proteins of the electron transport chain in mice, led to adipocyte death and inflammation in white adipose tissue (469), backing up the idea of mitochondria behind the inflammatory changes in adipose tissue. While it is still not established whether inflammation or mitochondrial function are first compromised in unhealthy obesity, our study, in line with previous studies (262) suggests that these two may be interconnected in a way that mitochondrial dysfunction may lead to decreased energy levels in the cell, apoptosis, cell death and in consequence, to the development of the inflammatory milieu. This inflammatory milieu in turn may also affect mitochondrial biogenesis. In humans, use of anti-inflammatory medications has been reported to improve glycaemic control in type 2 diabetes and obesity (470). Future treatments for the metabolically unhealthy obese might thus include agents that improve mitochondrial function, reduce or inhibit the formation of inflammation in adipose tissue or enhance the differentiation or expansion potential of preadipocytes in adipose tissue.

Interestingly, it is possible that the MHO stage may not be a lasting phenotype, but will change with age or with advanced obesity (418, 419). The hyperplastic capacity of adipose tissue has been shown to be reduced with age (471-473) and this might be one factor behind the change. In our study however, the two metabolically distinct groups were of the same age and had similar age of onset of obesity discordance. Weight differences between the groups were similar. An interesting fact however is that a given weight difference may have different metabolic effects depending on where in the distribution of the BMI a twin pair is located. A 5 unit increase from the BMI of 23 may have different metabolic effects than a 5 unit increase from the BMI 27. However, it may be speculated that in different individuals, depending on their adipose tissue buffering capacity, the metabolically unhealthy obesity is reached at different levels and/or different ages of obesity. In the general population a liver fat percentage of 5% has been considered a cutoff for “normal” liver fat content. All of the lean twins in our healthy MZ twin cohort do fall below this 5% cutoff. However, while the difference in liver fat content between the obese and the lean co-twins in group 2 is striking, not all “obese” co-twins have liver fat values exceeding 5%. It is of note that the differences between the co-twins are significant and consistent, suggesting that even in obesity that has lasted for a

relatively short time (6±years), the early changes of unwanted metabolism are striking, especially in the metabolically unhealthy obesity group.

Based on our findings, we conclude that the MHO phenotype was characterised by the maintenance of mitochondrial function and absence of inflammation in SAT. The metabolically unhealthy obese in turn had fatty livers and the hallmarks of the metabolic syndrome, impaired glucose tolerance and dyslipidaemia. Also, fitting to study I, the adipose tissue of the MHO individuals was characterised by the capability for both hypertrophy and hyperplasia of adipocytes with increasing BMI.

7.4. Mitochondrial oxidative pathway downregulation in acquired obesity in adipose tissue and adipocytes and the metabolic complications in whole body

The novel findings in studies III and IV were the downregulation of mitochondria-related pathways, their upstream regulators, mtDNA amount, mtDNA transcripts and OXPHOS protein subunit levels. Downregulation was shown in a wide spectrum in many analyses of mitochondria in adipose tissue in article III, and the transcriptional downregulation confirmed in adipocytes in article IV. Many of the differentially expressed transcripts were also differentially methylated between the co-twins in adipose tissue and the methylation in general inversely correlated with the expression of the gene, possibly suggesting a role of epigenetic modification in the reduction of mitochondrial biogenesis in obesity. Reduction of the mitochondrial oxidative metabolism in SAT correlated with whole body insulin resistance and inflammation in adipose tissue and adipocytes. We were also among the first to show a downregulation of OXPHOS subunits of complexes I and IV in obese adipose tissue, a finding which was then published also by Fischer et al 2015. We also confirmed reduced levels of mitochondrial encoded subunits of complexes and III and V, as well as nuclear-encoded complex II. We used a unique study design of rare young and clinically healthy MZ weight-discordant twin pairs, which enabled us to control for sex, age, and genetic background between the lean and the obese groups. Thus, we could establish these mitochondria-related changes as a consequence of acquired obesity, not genetic influence. Our findings provided evidence that a substantial insufficiency of mitochondrial biogenesis in SAT in acquired obesity occurs already prior to the onset of clinical metabolic complications.

The mitochondrial OXPHOS system is unique because it is encoded by both the nuclear and the mitochondrial genomes. In study III we showed that mtDNA amount, its transcript levels, OXPHOS subunit levels as well as nuclear genes encoding OXPHOS protein subunits are reduced in the obese co-twins suggesting a major downregulation in mitochondrial oxidative ATP production and catabolic functions in SAT in acquired obesity. Previous studies by us (262) and by others (251) have found similar reduction of mtDNA amount in SAT in obesity, but the generalized reduction of mitochondrial protein subunit levels and the downregulation of oxidative metabolism have not been reported previously. The OXPHOS complexes partially encoded by mtDNA were downregulated without significant decrease of mitochondrial mass (shown by the ratio of mitochondrial membrane protein porin to cytosolic β-tubulin), showing that the mitochondrial OXPHOS subunit levels are reduced in SAT both within the cell and within the mitochondria in the obese co-twins. The

translation machinery of mitochondria was shown to be downregulated by both the nuclear-encoded and the mitochondrial-encoded genes as the rRNAs and MRPs were both downregulated in the obese co-twins. Our findings are in agreement with earlier studies on the subject, where in two mouse models of obesity and type 2 diabetes a low abundance of MRP transcripts and reduced mitochondrial biogenesis in inguinal adipose tissue was observed (67), as well as in a rat model for late-onset obesity, where a truncation of MRPS26 was associated with the accumulation of visceral fat and insulin sensitivity (474).

Pathway analysis of nuclear-encoded mitochondrial transcripts revealed reduced expression of mitochondrial oxidative pathways, including FAO, TCA cycle, ketogenesis, ketolysis, and BCAA degradation in the obese co-twins. These mitochondrial pathways correlated negatively with several clinical parameters including all adiposity measurements, insulin resistance and inflammation, and positively with adiponectin levels. Mitochondrial metabolism and the development of type 2 diabetes have been thought to be linked (475). Studies in adipocytes or adipose tissue in human subjects are however few. 3T3-L1 mouse preadipocytes have been exposed to high glucose and fatty acids, resulting in signs of mitochondrial dysfunction by decreased mitochondrial size, membrane potential and downregulation of the master regulator *PGC-1α* (476). High-fat feeding has resulted in decrease of *PGC-1α* expression, impaired glucose homeostasis and reduced mtDNA content in rats (105). However, another study on mouse adipocytes did not register a relationship between mitochondrial biogenesis and glucose homeostasis (477). In studies III and IV we however confirm that reduced mitochondrial biogenesis, at least at the transcript level, is related to disturbances in insulin sensitivity both in human adipocytes and adipose tissue. What was remarkable was that the observed changes occurred already at a relatively young age, in twins that were clinically healthy, but where still a slight but significant worsening in the metabolic parameters in the obese co-twins could be observed.

We have previously reported downregulation of transcripts related to BCAA catabolism in SAT in obesity (262). Also in studies III and IV we observed the downregulation of BCAA catabolism in obesity and in study II of this thesis for the first time showed it to be restricted to the unhealthy group 2, with increased liver fat and other metabolic abnormalities. This was a new and important finding. BCAAs, especially leucine, stimulate adipocyte growth and differentiation through mTOR pathway (478). Decreased adipose BCAA catabolism has been shown to lead to a rise in plasma BCAA levels in obesity (165) and associated with the development of type 2 diabetes (264). However, it is still controversial if the observed rise in plasma BCAAs is a cause or a marker of the development of insulin resistance and metabolic complications of obesity (167). A study by Su et al showed that the intermediary metabolites of BCAAs (monomethyl BCAAs) accumulate in adipose tissue in obesity, and that this accumulation is linked to the obesity-related insulin resistance (444). The accumulation of the metabolites occurs if adipose tissue mitochondrial oxidation is impaired. Weight loss after gastric bypass has shown to increase the levels of BCAA oxidation enzymes in adipose tissue and as a result, the circulating BCAA levels decline (166). In our study of MZ twins, plasma levels of BCAAs were indeed elevated, while the pathway analyses indicated a decreased catabolism of BCAAs in mitochondria (479). Adipose tissue however is not the only site of BCAA catabolism, and also temporal differences in the catabolism rate are not known. The elevation of the circulating BCAAs in obesity has been known since the 1960s, but the results (262) from recent years for the first time link this to the decreased catabolism of BCAAs in mitochondria of obese individuals. In study II of the thesis, a significant decrease in the expression of the genes required

for this BCAA catabolism was present only in the adipose tissue of the obese twins of group 2, with increased amounts of liver fat and with metabolic impairments.

While mitochondria are essential in adipogenesis (6) and the same transcription factors are needed for both mitochondrial biogenesis and adipogenesis (133, 145), it is reasonable to think that defects in each of these factors might affect the other. Indeed, while both reduced hyperplastic capacity and reduced mitochondrial function of adipose tissue seem to be linked to the metabolic problems of obesity, the dysfunction and low amount of mitochondria in adipocytes could be suggested to be one factor behind the hyperplastic capacity itself. This could play a role in determining at which stage the adipose tissue dysfunction in obesity emerges and whom it concerns at the individual level, possibly distinguishing the metabolically healthy and unhealthy obese persons from each other. It is not known if the mitochondrial oxidative capacity is genetic and the hyperplastic capacity of adipose tissue thus also genetic, or the other way around. Based on study I, we showed that at least the regulation of adipocyte number in twins seems to be genetic. Concerning the analyses in this issue of the metabolically healthy and unhealthy obesity, the protein level analyses on 7 twin pairs and transcriptomics in 12 twin pairs was not sufficient to separate metabolically healthy or unhealthy twin pairs, because not enough samples were available from the unhealthy pairs. The reduction in mitochondrial oxidative metabolism in adipose tissue and adipocytes seems to be caused by the acquired obesity, based on the difference in mitochondrial biogenesis between the obese and the lean identical co-twins. For future analyses an interesting question to consider is: are there interindividual differences in the oxidative metabolism of mitochondria in an energy overload state, prompting some obese persons to develop complications and "the metabolically unhealthy obesity" earlier than others?

An interesting new finding in article III was the relationship of DNA-methylation to mitochondrial biogenesis. Our data showed that 74 out of the 222 differentially expressed genes targeting mitochondria were differentially methylated between the co-twins, and in general inversely correlated with gene expression. We also showed that three CpG sites in the body of mitochondrial regulator PGC-1α were hypermethylated in the obese co-twins. This hypermethylation correlated negatively with gene expression, suggesting that methylation may reduce the transcription of the gene. Previously it has been reported that compared with the lean controls, obese patients with type 2 diabetes have a PGC-1α promotor hypermethylation in SAT (480). Thus, it is possible that epigenetic modifications in certain nuclear genes associate with the decrease in mitochondrial biogenesis in SAT in acquired obesity. In obesity-discordant MZ twins, such an effect seems to be acquired, independent of the genetic background.

In article IV the downregulation of mitochondria-related pathways and upregulation of inflammatory pathways was observed in two independent analyses, both in examining the most significantly differentially expressed genes and their pathways between the co-twins as well as by comparing the genes that were consistently up- or downregulated between the co-twins. The small number of twins in article IV could however be a reason for the smaller amount of significant transcripts in adipocytes after multiple correction than was observed in article III for adipose tissue. Our results however reinforce the idea that the transcriptional changes seen in obesity are both consistent and involving many networks and many cells, most importantly the adipocytes themselves. We could also demonstrate the reduction of the mitochondrial mtDNA transcripts in adipocytes as well as a reduction in the expression of the main mitochondrial regulator *PGC-1α*. In

unrelated individuals, Western Blot of OXPHOS proteins subunit levels were reduced in the obese individuals in complexes CI (NDUFB8) and CIII (CIII-core 2 subunit) compared to cytoskeletal actin. The levels of complex IV and V subunits were lower but non-significantly so in the obese individuals. The low significance in the results was probably due to the very small sample size. Compared to mitochondrial membrane protein porin, CI subunit NDUFB8 was shown to be reduced in the obese individuals and levels of CIV subunit COX2 tended to be reduced. According to these data it seems plausible that in adipocytes and adipose tissue there is a shutdown of mitochondrial biogenesis and/or reduction of the levels of mitochondrial components in the obese state and that similar pathways are responsible for this. However, the studies on protein levels may need to be confirmed with larger data. It remains to be established whether there is a difference in the mitochondrial component levels, function and biogenesis in the small and large cells from obese compared with lean persons. Pre-adipocytes differentiated from human subcutaneous adipose tissue of obese persons have been shown to have lower oxygen consumption rates after beta-adrenergic stimulation compared with pre-adipocytes from lean subjects (68). However, in other studies no difference has been found between the oxidative capacities of small and large adipocytes (10, 235). Further studies on cultured adipocytes seem to be a relevant approach to clarify this issue.

Strong upregulated inflammatory gene expression emerged both in adipocytes and adipose tissue of the obese co-twins. Obese adipose tissue is characterized by accumulation of macrophages and other immune cells (228, 481). The current literature however seems to be controversial whether or not the inflammatory component seen in obese adipose tissue originates from the inflammatory cells of the tissue or adipocytes themselves. Some studies suggest that SVF cells and infiltrating macrophages are responsible for the cytokine secretion in adipose tissue (310, 482). Adipocytes are known secretors of IL-6, IL-8, TNF-α and other inflammatory adipokines (311), and exhibit immune cell-like functions that trigger CD4 T cell inflammation in the tissue, independent of infiltrating macrophages (483). Macrophage-derived TNF-α has also been shown to upregulate the expression of inflammatory genes like ICAM-1, IL-6 and MCP-1 in adipocytes (299, 309) and in obese adipocytes, inflammatory gene expression is shown to be increased (231). Adipocytes also express high levels of inflammatory CD14 as well as other previously thought to be macrophage-markers such as CD68 (484) on their surfaces, speaking for their role in the mediation of inflammation. The disagreement seems to be whether or not the adipocyte-derived factors have clinical significance compared to the macrophage and other inflammatory cell-derived ones. A study involving arteriovenous examination of TNF-α flow from human subcutaneous adipose tissue showed that TNF-α is not secreted and mobilized systemically, but IL-6 instead is (485). However, the technique used in the study may not have been able to detect low levels of TNF-α. Also, the amount of macrophage-derived or adipocyte-derived inflammatory factors may differ. The upregulation of inflammatory genes and pathways in adipocytes of the obese co-twins of our study IV suggests that adipocytes do contribute to the inflammatory milieu of obese adipose tissue, and that obesity induces changes also in the adipocyte-derived inflammatory component of obesity.

One hypothesis behind the observed changes could be that in an excess energy state, the cell shuts down its mitochondria while producing energy more easily and effectively in cytoplasm by anaerobic glycolysis. The mitochondria of adipocytes however may be stressed from excess free fatty acids and energy substrates, releasing more free radicals that derange the cell metabolism, enhance inflammation and mitochondrial function and finally, the rate of oxidative energy production further declines. How the mitochondrial respiration is affected in the metabolically

healthy and unhealthy subjects will be an interesting issue of future studies. Adipose tissue is an especially challenging research material because there are few mitochondria in adipocytes and thus a low mitochondrial yield in white adipose tissue. The same is true for adipose tissue protein yield, RNA yield and many other methods. This could be one reason behind such few publications in the issue.

In summary, in articles III and IV we observed strong evidence for association between acquired obesity and downregulation of mitochondrial biogenesis both in adipose tissue and adipocytes. In adipose tissue, the genes important for mitochondria may also be epigenetically regulated. Importantly, we showed that the downregulation of mitochondrial biogenesis and oxidative metabolic pathways in subcutaneous adipose tissue and adipocytes is a phenomenon in obesity that on transcript level is associated with mild metabolic alterations, insulin resistance, and low-grade inflammation.

Figure 21

Figure 21A: Adipose tissue dysfunction and reduced mitochondrial biogenesis in obesity

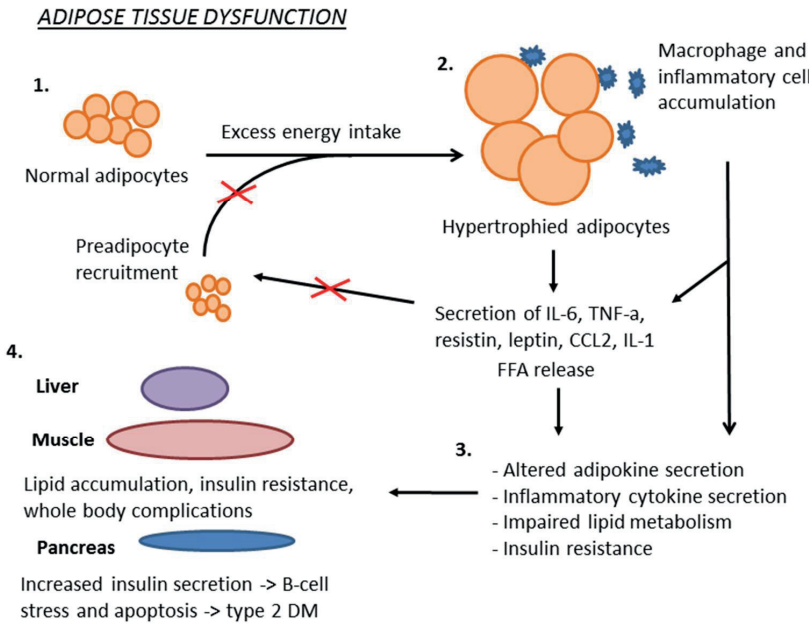


Figure 21A: *Excess energy intake leads to hypertrophied adipocytes. This is thought to lead to the accumulation of inflammatory cells in the tissue. However, the mechanisms have been unclear. Increased secretion of inflammatory markers and increased FFA release from hypertrophied adipocytes prevents preadipocyte recruitment and impairs lipid and glucose metabolism in adipose tissue and consequently, in the whole body. Intestines and other organs like liver, muscle and pancreas accumulate lipids and this leads to their impaired function and insulin resistance.*

Figure 21B: Adipocyte dysfunction in obesity

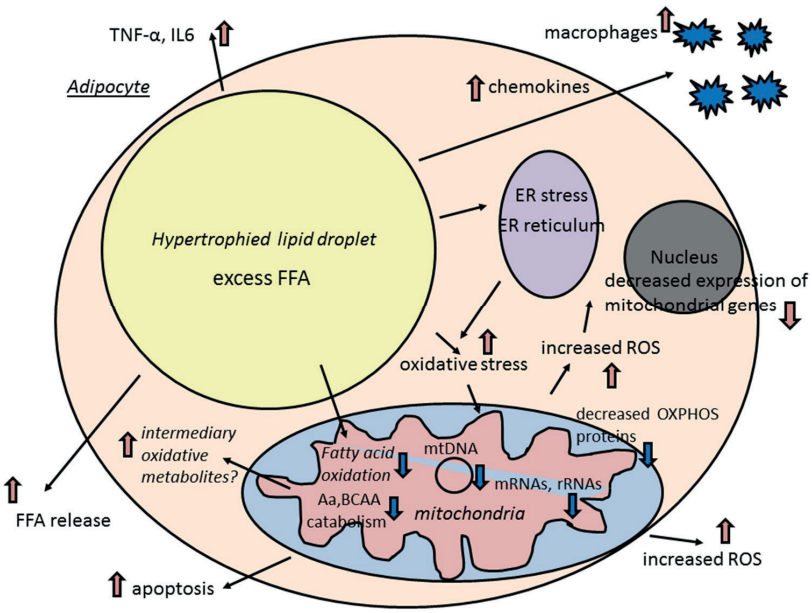


Figure 21B: *Excess FFA and adipocyte stress -> reduced mitochondrial biogenesis? Hypertrophied adipocyte lipid droplet may endorse endoplasmic reticulum stress, oxidative stress and stress for the adipocyte cell membrane. Adipocytes secrete increased levels of inflammatory markers. The changes in the adipocyte membrane structure due to cell stress and expansion recruit the macrophages and other inflammatory cells in the tissue. When adipocyte is not able to store all the incoming fat, part of the fat is flown to ectopic sites. FFA release from the cell increases contributing to hyperlipidemia in obesity. In mitochondria, fatty acid oxidation and BCAA oxidation become impaired with the overflow of FFAs. Increased amounts of FFAs that cannot be oxidized are also released as intermediary metabolites that can disturb the function of the cell. Markers of oxidative stress and ROS production from mitochondria increase. This in turn increases the adipocyte stress reaction. Nuclear expression of mitochondria-related regulator genes and pathways is downregulated. Whether this is due to the adipocyte stress reaction or the cell shuts down its mitochondria in favor of oxygen-independent metabolism of FFAs, or from other reasons, is still unknown and may be a vicious circle.*

Figure 21C: Reduced mitochondrial biogenesis in adipose tissue and adipocytes in obesity

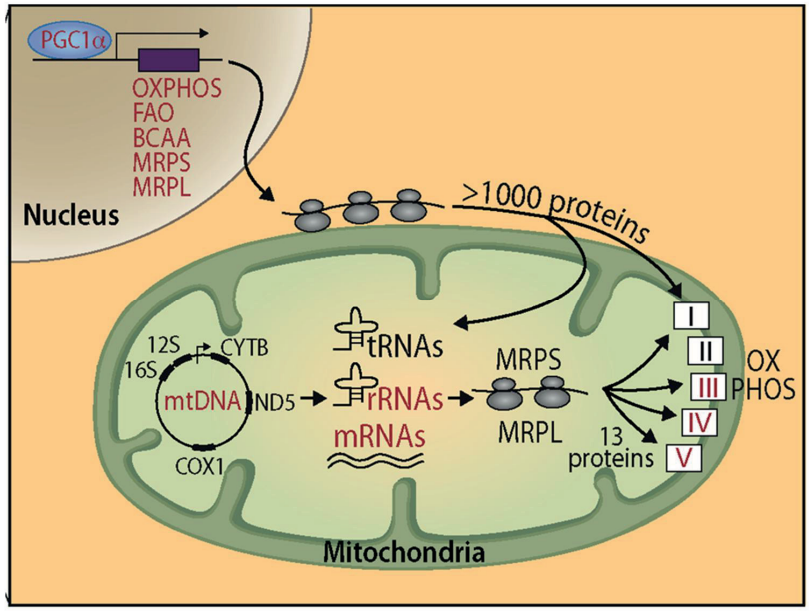


Figure 21C*: Reduced expression of the main mitochondrial regulator PGC-1α (qRT-PCR) and reduced expression of the main mitochondrial pathways; oxidative phosphorylation (OXPHOS), fatty acid β-oxidation (FAO), branched-chain amino acid oxidation (BCAA) in adipose tissue and adipocytes. Reduced levels of mitochondrial mtDNA (adipose tissue), mtDNA transcripts (rRNAs and mRNAs), the nuclear expression of mitochondrial ribosomal protein subunits (MRPS, MRPL) and levels of oxidative phosphorylation (OXPHOS) protein subunits in obesity (Western Blot) in adipose tissue and adipocytes.*

7.5. Methodological considerations

Because of the extreme rarity of the MZ twin pairs, the study samples were small. A different amount of twin pairs were available for the four sub-studies and their laboratory experiments. Also, our sample of concordant pairs in studies I and IV (n=5) is also small. Study IV included 14 discordant twin pairs, from which enough sample for adipocyte extraction was available. Results obtained from them may thus be interpreted with caution. Further, there was a small but statistically significant difference in the BMI of the weight-concordant pairs in articles I and IV. We also did not report results on DZ pairs, and formal calculations of heritability cannot therefore be performed. Analyses on adipose tissue included also stromal vascular fraction (SVF) cells, which contribute to the results. Adipocyte extraction and purification protocol for separating adipocytes from the tissue matrix is a common and widely used protocol, but the exact level of adipocyte purification from SVF cells could not be assessed. Assessing the cell size was done by calculating the cell diameters with a light microscope, by which the populations of very small cells may have remained undetected. Thus, a lower limit for the adipocyte cell size (20 µm) was set and the populations of very small cells were not addressed in these studies. Transcriptomic data may not fully reflect the real protein levels and further their activity in the tissue and thus additional studies on protein level may be needed to confirm part of the results. Data analyses and the analysis of PCR experiments were done with different methods between the sub-studies, however all yielding biologically significant and consistent results. Because of the cross-sectional nature of the study, we cannot make cause and effect conclusions. The nature of the study data is also associative. However, our findings were in line with many previous studies on obesity and adipose tissue.

7.6. Future prospects

The results of the thesis as a whole suggest that the metabolic complications of obesity could be reduced by enhancing mitochondrial amount or function in white adipose tissue and that this might improve whole-body insulin sensitivity. Also, diminishing inflammation or enhancing the preadipocyte capacity for differentiation in subcutaneous adipose tissue might prove to be beneficial.

Some pharmacological interventions that have already been tested to treat insulin resistance might be of use also in obesity. Thiazolidinediones (TZDs), which are PPARγ agonists, have been used to treat patients with type 2 diabetes, because they improve the insulin sensitivity of the white adipose tissue. However, there is evidence that TZDs also increase mitochondrial biogenesis, function and content. Furthermore, TZDs improve palmitate-stimulated oxidative capacity in adipocytes in mice, and upregulate mitochondria-associated genes and PGC-1α, one of the main mitochondrial regulators (66, 142). In humans, evidence of this connection has also been shown *in vitro* (103) as well as *in vivo* where treatment with pioglitazone improved mitochondrial biogenesis in diabetic patients by stimulating the expression of genes important for fatty acid oxidation and PGC-1α, as well as by increased mitochondrial copy number (486). Thus, increasing mitochondrial biogenesis by thiazolidinediones might be of use in obesity too. However, for example rosiglitazone from the same group of compounds has caused cardiovascular problems like heart failure and death - reasons why it was withdrawn from use. In humans, mitochondrial antioxidant treatment with vitamin D (487), vitamin E (488), vitamin C, N-acetylcysteine, glutathione and coenzyme Q10, may improve

insulin sensitivity in diabetic patients (489). R-alpha-lipoic acid (LA) and acetyl-L-carnitine (ALC) have been shown to enhance mitochondrial function in cultured mouse adipocytes (490). However, other good-quality human studies have failed to show the association between antioxidants and improved metabolism (491, 492). Chemical uncouplers that dissipate the energy produced by mitochondria as heat (493) have been tried as dietary drugs. Weight losses were recorded (494), but this uncontrolled thermogenesis also lead to fatal increases in body temperature (495) and the idea was discontinued. So far, exercise and caloric restriction remain established improvers of mitochondrial biogenesis and function. NAD+-dependent deacetylase sirtuin 1 (SIRT1) is suggested to be the principal modulator of caloric restriction (496, 497). Resveratrol and other small molecule compounds that activate SIRT1 have increased mitochondrial respiration as well as produced an improvement in insulin sensitivity in mouse studies (498, 499).

In rodent studies, knocking out inflammatory genes prevented the development of type 2 diabetes (304, 500) and in humans, the use of anti-inflammatory medications improves glycaemic control in type 2 diabetes and obesity (470). Thus, preventing inflammation in adipose tissue might prove to be beneficial in treating and preventing obesity-associated disorders. Inflammation in adipose tissue has been targeted for example by amlexanox, an inhibitor of the innate immune system, which improves insulin sensitivity, promotes weight loss and reduces WAT inflammation and hepatic steatosis in mice (501). Amlexanox has also been in phase II clinical trials for obese patients with type 2 diabetes (US National Library of Medicine, ClinicalTrials.gov, http://www.clinicaltrials.gov/ct2/show/NCT01842282, 2013) and an improvement in HOMA–index has been preliminary reported (502). Other ways of targeting inflammation have included for example immunoneutralization of TNF with etanercept, which lowered systemic inflammatory markers in obese diabetic patients (503), and interventions that block macrophage chemotaxis into WAT, which had insulin-sensitizing effects (504). Treatment of mice with an inhibitor of the pro-inflammatory eicosanoid leukotriene B4 (LTB4) resulted in an anti-inflammatory phenotype and increased insulin sensitivity as well as decreased hepatic steatosis (505). Of all the compounds mentioned, the LTB4-targeted compound has been validated in one human clinical trial in healthy males (506); however, not in the context of obesity and type 2 diabetes.

Some of the newest discoveries in adipose tissue-related diseases suggest that in addition to increasing mitochondrial biogenesis and function or decreasing inflammation, novel therapeutic approaches may include adipokine-based therapies, promotion of white fat beiging (browning), and modulation of fibrosis and the oxidative state of the adipose tissue. Adipokine-related therapies like administration of leptin-recombinants or activation of adipokine receptors have recently harboured some new discoveries. Fibroblast growth factor 21 (FGF21), bone morphogenic protein 7 (BMP7) and interleukin 1 beta (IL-1 β) are under research for their use in the treatment of obesity. Brown fat activation and white adipose tissue "beiging" might also prove beneficial, because BAT is important in cold- and diet-induced thermogenesis and dissipates energy to generate heat by uncoupling mitochondrial respiration (507). Beige cells, mingled in white adipose tissue, have been in mouse studies shown to have potential to be activated and their amount increased by cold exposure, exercise training and possibly by specific pharmacological compounds (508). However, the compounds tried for BAT activation and WAT browning have suffered from various side effects like hypertension and cardiovascular effects (44). In mice studies, hypoxia-inducible factor HIF1α inhibitors have

increased energy expenditure and reduced body weight as well as adipose tissue fibrosis and inflammation (509). Angiogenesis inhibitors in obese adipose tissue have also reduced body weight (510) or prevented the development of obesity in mice (511, 512). However, healthy angiogenesis is also needed for energy expenditure (280, 284) and it is thus unclear if increasing or decreasing adipose tissue angiogenesis has beneficial effects on treating obesity.

Obesity is a multisystemic and multifactorial disease. Personalized therapies will be needed to treat obese with different metabolic status and to administrer drugs locally (44). Our data supports the findings that increasing mitochondrial metabolism, decreasing inflammation and enhancing preadipocyte differentiation capacity in adipose tissue will have beneficial effects in the treatment of obesity.

8. SUMMARY AND CONCLUSIONS

Background and aims

Obesity with its overload of adipose tissue is a health risk predisposing to many chronic diseases such as type 2 diabetes, cardiovascular diseases and cancers. Positive energy balance leading to accumulation of excess body fat and its complications often arises from a complex interplay of hereditary and environmental factors. Disentangling these two is difficult. The aim of this thesis was to study, in a unique group of young weight-discordant MZ-twins allowing the control of genetic factors, the biological pathways in adipose tissue that lead to the development of metabolic complications in obesity. We studied adipose tissue enlargement, one of the primary features in acquired obesity, by assessing adipocyte hypertrophy and hyperplasia and their effects on adipose tissue and whole body metabolic health. We investigated the mechanisms and pathways maintaining the "metabolically healthy obesity" phenomenon, where despite increased fat mass, certain obese individuals stay free from the metabolic complications of obesity. Based on the mitochondria-related findings we further concentrated on mitochondrial biogenesis in obese adipose tissue and its relationship to metabolic health in adipose tissue and in whole body by assessing the expression of mitochondria-related nuclear-encoded transcripts, mitochondrial DNA amount, mitochondrial DNA transcript levels and mitochondrial respiratory chain protein levels in obese adipose tissue and their associations with adipose tissue metabolism. Finally, we aimed at clarifying the status of mitochondrial biogenesis in purified adipocyte cells of the obese co-twins.

Subjects and methods

Altogether 26 rare BMI-discordant (intra-pair difference (Δ) in BMI 3-10 kg/m^2 and 14 concordant (ΔBMI 0-2 kg/m^2) young healthy adult (22-36 years) monozygotic (MZ) twin pairs were studied. The subjects were recruited from FinnTwin16 1975-1979 (FinnTwin16, n=2 839) and FinnTwin12 1983-1987 (FinnTwin12, n=2 578) registries. The twins underwent an extensive study protocol with measurements of body composition, body fat distribution of subcutaneous and visceral adipose tissue and liver fat, oral glucose tolerance test (OGTT), lipids, adipokines, hs-CRP and other metabolic measurements from blood samples, adipose tissue biopsies under the umbilicus and many questionnaires and interviews. Total RNA from both adipose tissue and adipocytes was extracted and transcriptomics analyses of fat in whole human genome were performed. Differences between the obese and the lean co-twins in pathways related to adipocyte morphology, metabolic disturbances, mitochondrial function and inflammation in subcutaneous adipose tissue were analysed. Adipocytes were photographed and calculated and the diameters of the cells were measured. Total DNA from adipose tissue samples was extracted and the amount of mitochondrial mtDNA determined. MtDNA-encoded transcript levels were measured in adipose tissue and adipocytes. Western blot of total adipose tissue and adipocyte lysates were done for oxidative phosphorylation (OXPHOS) complexes I, II, III, IV and V. Clinical measures and blood sample data were correlated with gene expression results.

Results

In the BMI-discordant MZ pairs, the obese co-twins had significantly more fat mass in their subcutaneous, visceral and liver fat depots and larger adipocytes, higher hs-CRP and plasma leptin levels, lower plasma adiponectin levels and presented with more insulin resistance than their leaner co-twins. However, the discordant twins resembled each other for adipocyte number, speaking for a tight genetic regulation of this measure. The BMI-concordant MZ pairs were similar in their body composition, fat distribution, as well as adipocyte size and number. In the discordant pairs, increased adipocyte size (hypertrophy) was related to downregulation of mitochondria-related gene expression pathways and increased cell death. Obese co-twins who had a more hypertrophic obesity profile (larger adipocytes in their adipose tissue) with a decreased cell count had, compared with their leaner co-twins, significantly more liver fat, insulin resistance, inflammation and LDL cholesterol. In contrast, obese co-twins who had less hypertrophy but an increased adipocyte count compared with their lean co-twins (hyperplastic obesity) were indistinguishable from their leaner counterparts in most metabolic measures. This indicates that the hypertrophic vs. hyperplastic obesity are metabolically different.

We were also able to divide the BMI-discordant twin pairs into two metabolic groups based on the amount of their liver fat. In half of the pairs the obese co-twins had as low liver fat percentages as their leaner co-twin, whereas in the other group the obese co-twins had a strikingly increased liver fat content compared with their leaner co-twins. The obese in the latter group were metabolically unhealthier than their peers in the first group despite the fact that these two groups did not differ in overall fatness. Gene expression analyses in adipose tissue revealed that the expression of mitochondrial pathways and chronic inflammatory response were increased, and adipocyte differentiation downregulated in the obese compared with the lean co-twin in the high liver fat group with metabolic problems, but not in the low-liver fat group.

Further analyses on mitochondrial biogenesis using adipose tissue of the twins revealed that a significant proportion of the genes that were significantly differentially expressed between the co-twins were related to mitochondria. Mitochondria-related pathways were downregulated in the obese co-twins, both for nuclear-encoded mitochondria-related genes gas well as for mitochondria-encoded transcripts. The subunit levels of mitochondrial oxidative phosphorylation (OXPHOS) protein complexes were reduced in the obese co-twins´ adipose tissue indicating a reduction of mitochondrial oxidative metabolism. In adipocytes of the obese co-twins, we observed similar transcriptional downregulation of mitochondrial biogenesis as in the whole adipose tissue.

Conclusions

Studies I and II of this thesis revealed two distinct metabolic groups of obesity, independent of genetic effects, with adipocyte hypertrophy/reduced hyperplasia and increased liver fat distinguishing the metabolically high risk group from the “healthy obese” group**.** While adipocyte number in the body seemed to have a strong genetic component, it may be postulated that the capacity to adipoce hyperplasia may be genetically determined. Increased adipocyte size and decreased number associated with the worsening of the metabolic profile in obesity with gene expression changes indicating downregulation of mitochondrial pathways.

We further found that, independent of genetic effects, there were distinct differences in the gene expression profiles between the obese and the lean MZ co-twins, with downregulation of mitochondrial biogenesis and up-regulation of inflammatory pathways playing central roles in the metabolic complications of acquired obesity. In study II these changes associated specifically to the obese twins with high liver fat content.

In study III the mitochondrial downregulation was evidenced by reduced expression of nuclear-encoded mitochondria-related genes and pathways, their upstream regulators, mitochondrial copy-number, mtDNA transcripts and finally on reduced OXPHOS protein levels. The same pattern of downregulation of mitochondrial gene expression pathways and genes and upregulation of inflammation was observed also in isolated adipocyte cells. In both studies, the mitochondrial downregulation associated with the metabolic disturbances of obesity.

The transcriptional changes observed in acquired obesity and their associations to whole-body metabolism could already be seen at a young age in clinically healthy twins with relatively short history of obesity, emphasizing the need for early detection and intervention of this disease and the detection of persons most vulnerable to the complications of obesity. According to the study, liver fat content and the capacity of adipose tissue to hyperplasia seem to be clear determinants of metabolic health in acquired obesity. The results of my thesis as a whole suggest that obesity-associated metabolic disturbances might be halted by improving mitochondrial activity in adipose tissue. While medications to stimulate mitochondria in humans are still lacking, caloric restriction and exercise thus far seem to be the only ways to improve mitochondrial function in adipose tissue, according to literature. Changes in lifestyle benefit all obese persons, because it is not know if the observed “healthy” obesity can over time turn to unhealthy obesity.

9. YHTEENVETO JA JOHTOPÄÄTÖKSET (in Finnish)

Tausta ja tavoitteet

Lihavuus eli liiallinen rasvakudos on merkittävä terveysriski ja aiheuttaa monia aineenvaihdunnan häiriöitä, kuten insuliiniresistenssiä, tyypin 2 diabetesta, sydänsairauksia, hyperlipidemiaa ja syöpää. Lihomiseen ja rasvakudoksen laajentumiseen liittyvät metaboliset ongelmat johtuvat usein geenien ja ympäristötekijöiden monimutkaisesta yhteisvaikutuksesta. Näiden tekijöiden erottaminen toisistaan on vaikeaa. Lihavuuden syistä ei ole edelleenkään kokonaisvaltaista ymmärrystä. Viime vuosina on kuitenkin huomattu, että rasvakudos ja erityisesti sen solujen energiatehtaiden eli mitokondrioiden toiminta on keskeisessä asemassa koko kehon aineenvaihdunnassa ja mahdollisesti myös lihavuuden kehittymisessä. Väitöskirjassani tutkin nuorten terveiden identtisten kaksosten rasvakudoksen varhaisia aineenvaihduntamuutoksia ja insuliiniresistenssiä hankitussa lihavuudessa. Tässä asetelmassa voidaan ainutlaatuisesti erottaa geeneistä ja ympäristötekijöistä johtuvat lihavuuden seuraukset.

Rasvakudos laajenee joko kasvattaen rasvasolujensa kokoa tai niiden lukumäärää. Väitöskirjassani tutkin, miten rasvasolujen koko ja määrä muuttuvat lihotessa ja mikä on näiden muutosten yhteys kehon koostumukseen, aineenvaihduntaan ja rasvakudoksen geenien ilmenemiseen. Tarkastelemme, mitkä geenien ilmenemisreitit ja muut kehon aineenvaihdunnan muutokset erottavat aineenvaihdunnaltaan terveitä lihavia sairaista lihavista. Saatujen tulosten pohjalta tutkimme tarkemmin rasvakudoksen mitokondrioiden ja niiden hengitysketjun toimintaa ja tämän yhteyttä kehon aineenvaihduntaan sekä selvitimme, millainen on mitokondrioiden toiminta rasvakudoksen rasvasoluissa.

Aineisto ja menetelmät

Tutkimuksen aineistona käytettiin identtisiä, mutta eripainoisia suomalaisia kaksosia. Aineisto on harvinainen, sillä geeneiltään identtisiä mutta painoiltaan erilaisia kaksospareja on vähän. Tutkimukseen osallistui yhteensä 26 diskordanttia eli eripainoista (ΔBMI 3-10 kg/m^2) ja 14 konkordanttia eli samanpainoista (ΔBMI 0-2 kg/m^2) nuorta suomalaista identtistä kaksosparia (ikä 22–36 vuotta). Tutkittavat kaksoset olivat FinnTwin16- 1975–1979 (FinnTwin16, n=2 839) ja FinnTwin 12- 1983–1987 (FinnTwin12, n=2 578) kohorteista löydettyjä vapaaehtoisia.

Kaksosparien kehonkoostumus, kehon rasvajakauma ja maksan rasva mitattiin. Veren paastosokeri, insuliini, rasvat, adipokiinit ja tulehdusmerkkiaineet määritettiin. Vatsan alueen ihonalaiskudoksesta kerättiin näytteet rasvakudoksesta. Rasvakudosnäytteistä sekä niiden rasvasoluista eristettiin RNA ja DNA. Kaksosparin geenien ilmentymistä ja sen eroja tutkittiin koko genomin laajuisesti (20 000 geeniä, Affymetrix U133 Plus 2.0 sirut) ja analysoitiin geenireittejä liittyen lihavuudessa havaittuihin metabolisiin häiriöihin, mitokondriotoimintaan, rasvasolujen määrään, kokoon ja rasvakudoksen tulehdukseen. Rasvakudoksen mitokondrio-DNA ja sen kopiolukumäärä määritettiin. Mitokondrion oman genomin ilmentymisen tasoja mitattiin qRT-PCR:llä ja mitokondrion hengitysketjun proteiinien tasot määritettiin Western Blotilla. Rasvakudosnäytteiden

rasvasolut valokuvattiin ja niiden koot ja muoto määritettiin. Useat kyselyt ja haastattelut kuuluivat lisäksi kaksosten perustutkimuksiin.

Tulokset

Eripainoisten kaksosparien lihavilla kaksosilla oli huomattavasti enemmän rasvamassaa, ihonalais- ja sisäelinrasvaa sekä maksan rasvaa kuin laihoilla kaksosilla. Lihavat olivat metabolisesti epäterveempiä; heidän insuliinitasonsa ja veren rasvatasonsa olivat korkeammat, rasvasolun kokonsa suurempi ja tulehdusmerkkiaineensa korkeammalla tasolla kuin laihoilla kaksosilla. Lihavien kaksosten kehon rasvasolumäärä ei kuitenkaan eronnut laihojen kaksosten vastaavasta, viitaten siihen, että rasvakudoksen solumäärä on vahvasti geneettistä perua. Verrokkipareina toimineilla konkordanteilla eli samanpainoisilla kaksosilla kaikki aineenvaihdunnan mitat ja veren tulehdus- ja muiden merkkiaineiden pitoisuudet olivat toistensa kaltaisia. Näyttää siis, että havaitut lihotessa tapahtuvat muutokset rasvasolujen lukumäärää mukaan lukematta ovat lihavuuden ja ympäristötekijöiden aikaansaamia, eivät geneettistä perua.

Suuri rasvasolukoko ja toisaalta koko kehon pieni rasvasolumäärä yhdistyivät lihavuuden aiheuttamiin aineenvaihdunnan ongelmiin, insuliiniresistenssiin ja geenireitteihin, jotka liittyivät heikentyneeseen mitokondrioiden toimintaan ja lisääntyneeseen rasvakudoksen solukuolemaan. Kaksoset voitiin jakaa kahteen eri ryhmään sen perusteella, oliko heidän rasvasolujensa lukumäärä laihaan kaksososapuoleen suhteutettuna suurempi vai pienempi tai yhtä suuri. Ne lihavat kaksoset, joiden rasvasolumäärä laihaan pariinsa verraten oli suurempi (hyperplastinen eli rasvasolumäärää lisäävä lihavuus) olivat metabolisesti selvästi terveempiä kuin ne parit, joilla lihavalla kokonaisrasvasolumäärä oli pieni (hypertrofinen eli rasvasolun kokoa kasvattava lihavuus). Totesimme myös, että maksan rasvapitoisuuden perusteella oli mahdollista erottaa kaksi metabolisesti erilaista lihavuustyyppiä, joissa toisessa lihavat eivät näyttäneen kärsivän lihavuuden liitännäisongelmista. Tässä ”metabolisesti terveen” lihavuuden – ryhmässä lihavilla maksan rasvapitoisuus oli pieni ja yhdistyi mitokondrioiden korkeaan aktiivisuuteen ja vähäiseen rasvakudoksen tulehdukseen. Kolmannessa väitöskirjan osatyössä näytimme, että lihavuuteen liittyvä mitokondrioiden huono toiminta ilmenee niin solun tuman koodaamien mitokondrioon liittyvien geenien luennan vähentymisenä, mitokondrion kopiolukumäärän pienenemisenä kuin mitokondrion itsensä koodaamien geenien luennan ja mitokondrion hengitysketjun proteiinien vähentyneinä määrinä. Nämä tulokset yhdistyivät koko kehon insuliiniresistenssiin ja matalan tason tulehdukseen. Väitöskirjan neljännessä osatyössä samanlainen mitokondriotoiminnan vähentyminen oli nähtävissä rasvakudoksen rasvasoluissa.

Johtopäätökset

Väitöskirjani tuloksissa voitiin sekä rasvasolujen suuren koon ja pienen määrän (artikkeli I) että maksan rasvan (artikkeli II) avulla erottaa kaksi lihavuuden ryhmää, jossa alhaisen maksan rasvapitoisuuden ja hyvän rasvakudoksen laajentumiskapasiteetin omaavat lihavat näyttivät olevan suojatumpia rasvan kertymisen aiheuttamilta komplikaatioilta. Nuorten terveiden ja identtisten mutta eripainoisten kaksosten rasvakudoksen geenien ilmentymisessä todettiin lihavalla kaksosella

mitokondrioiden toiminnan vähentyminen ja tulehduksen lisääntyminen. Nämä tulokset yhdistyivät koko kehon huonontuneeseen aineenvaihduntaan ja insuliiniresistenssiin.

Mitokondrioiden vähentynyt toiminta näkyi rasvakudoksessa sekä tuman geenien että mitokondrion omien geenien luennassa ja todettiin myös mitokondrion hengitysketjun proteiinien vähentyneinä määrinä artikkelissa III. Artikkelissa IV sama mitokondriotoiminnan väheneminen, tulehduksen lisääntyminen sekä mitokondrion hengitysketjun proteiinien vähentyneet määrät osoitettiin myös rasvasoluissa. Tulosten perusteella rasvakudos ja sen rasvasolut sekä rasvasolujen mitokondrioiden toiminta näyttävät olevan tärkeä tekijä lihavuuden aiheuttamien ongelmien synnyssä.

Merkittävää tutkimuksessa oli, että geenien ilmentymisen muutokset olivat näkyvissä jo lihavuuden kestettyä vasta verrattain lyhyen aikaa kliinisesti terveillä nuorilla kaksosilla. Koska hankitun lihavuuden aiheuttamat haitalliset muutokset kehon geenien luennassa ja aineenvaihdunnassa ilmenevät nähtävästi jo hyvin aikaisin, on tärkeää pyrkiä tunnistamaan muutokset ja epäterveelle lihavuudelle alttiit henkilöt jo varhain. Maksan rasvapitoisuuden perusteella voidaan mahdollisesti erottaa lihavat, jotka ovat alttiita lihomisen aiheuttamille komplikaatioille ja insuliiniresistenssille. Väitöskirjani tuloksien perusteella lihavuuteen yhdistyviä metabolisia häiriöitä voitaisiin todennäköisesti estää lisäämällä ja parantamalla mitokondrioiden toimintaa rasvakudoksessa. Koska mitokondrioiden toimintaa lisäävien lääkkeiden kehitys on vasta varhaisvaiheessa, liikunta ja energiansaannin rajoittaminen ovat tähän mennessä kirjallisuuden perusteella ainoita toimivia keinoja parantaa mitokondrioiden toimintaa rasvakudoksessa. Elämäntapamuutokset hyödyttävät kaikkia lihavuudesta kärsiviä, sillä tutkimuksessa havaittu ”terve” lihavuus saattaa myös ajan myötä kehittyä epäterveeksi lihavuudeksi.

ACKNOWLEDGEMENTS

This work was carried out as part of the TwinFat 2008 study at the Obesity Research Unit, Diabetes and Obesity Research Programs Unit, University of Helsinki and DPBM Doctoral Program in Biomedicine, Helsinki Biomedical Graduate School, where I participated in the MD/PhD program. In addition, this study has been financially supported by Orion Pharmos Foundation, Biomedicum Helsinki Foundation, Finnish Medical Foundation, Maud Kuistila Foundation, Diabetes Research Foundation, Emil Aaltonen Foundation, Paulo Foundation and Ida Montin Foundation.

First of all, I want to express my warmest thanks and gratitude to my supervisor, Associate Professor ***Kirsi Pietiläinen***. She has enthusiastically guided me through this whole process and I have always been able to count on her advice, however small the subject, and support. She is one of the leading scientist in the obesity research field. She has enormous energy, interest and enthusiasm for her work and is deeply interested in the mechanisms behind obesity. Kirsi has provided me with interesting research topics and new ideas for these, and taught hand-in-hand statistics and the methodological analyses of my research. Most of all, she has been the most encouraging supervisor I could ever wish for, always giving good and reconstructive feedback, always happy about the work that I had done but still encouraging me to go even further. I also want to deeply thank my other supervisor, Professor ***Aila Rissanen***, who first met me in the summer of 2008 just before the start of my medical studies and asked me to work for her unit because she thought me of possessing intelligence and value. From then on, she has provided invaluable guidance and warm support in my scientific work as in other aspects of life. She has also given her personal time to teach me in fluent scientific writing and been always the one final reader for a manuscript; the one who makes sure everything in the texts is written the best possible way.

The extraordinary opportunity of using twins in my research has been possible thanks to Professor ***Jaakko Kaprio*** who has provided us with the large and internationally recognized datasets of Finnish twins that enabled this research. He has also been of great support for statistical analyses in all of my articles as well as providing help in how to use and analyze the twin data.

The reviewers of my thesis, Professor ***Eero Mervaala*** and Professor ***Olavi Ukkola*** have given their expertise and thorough efforts to improve this thesis. Your comments and suggestions have granted the thesis more clarity and precision. I most warmly thank you both for your excellent work. I also want to thank Docent ***Henna Tyynismaa*** and Professor ***Eero Mervaala*** for their support and work in my Thesis committee.

I would especially want to thank Professor ***Anu Wartiovaara*** for her collaboration and knowledge on mitochondria that has been so useful for this work as well as for her letting me to work in her laboratory during my first PhD years. Thank you also for acting as my Custos in the Public examination of this Thesis. And thank you to the laboratory experts of the 5th floor Anu Wartiovaara lab, ***Anu*** and ***Markus***, from whom I in my first years in lab could always ask for help.

I would like to thank all the co-authors of the four articles of this thesis for their thorough work in acquiring the data as well as in its analyses. I would further like to thank our whole research team,

the current as well as all the previous members of it, and all the many helpful and wonderful people that have been involved in teaching me and giving their expertise for the works presented here. Thank you ***Mia Urjansson, Katja Sohlo, Sari Räsänen, Tarja Hallaranta, Elisabeth Rappou, Riikka Jokinen*** and thank you ***Sanna Kaye*** and ***Leonie Bogl*** for your help and support as co-workers. Thank you ***Miina Ollikainen***, who helped me with some of my laboratory work in my first years as a PhD student and later with her epigenetic knowledge, as well as ***Jussi Naukkarinen*** for your guidance in the beginning of my studies. Thank you especially ***Jana Buzkova*** from the Anu Wartiowaara lab for your expertise on mitochondria that you generously shared with me and taught me many invaluable laboratory techniques. Thank you ***Mia Urjansson*** for help with the Western Blot analyses in the last article of my thesis.

I would also especially want to thank Professor ***Gema Frühbeck*** and Professor ***Peter Arner***, who gave me the possibility to come and visit their laboratories and research groups and learn many invaluable techniques for the laboratory work in adipocytes and adipose tissue. The whole laboratory staff in the Metabolic Research Laboratory in Pamplona and the Lipid Laboratorium in Karolinska Institutet gave me their time and expertise for free and unlimited, and for this I am very grateful. From you I learned the basic laboratory skills that I was then able to develop further. Especially I want to thank you ***Amaia Rodríquez***, for that you teached me hands on first here in Finland and then in Pamplona. With you I got the first experience of scientific laboratory work and learned the basics of it. You are a very good teacher, a dear friend and a collaborator. I want to thank Associate Professor ***Gijs Goossens*** from the NUTRIM School of Nutrition and Translational Research in Metabolism, Department of Human Biology, Maastricht University, The Netherland who kindly accepted the role of my opponent of the thesis in its Public Defence, and whose work has inspired me with new ideas on studies in the obesity and adipose tissue field. Lastly, I want to thank ***Eija Pirinen*** for sharing her knowledge on mitochondria and helping with proofreading the 3rd article of the thesis as well as the thesis book to check the consistency and accuracy of data in this aspect.

My friends have been of great support in other aspects of my life as well as encouraging me with the work during the whole PhD process. Thank you extremely much for it; you are all very dear and important to me!

I owe special thanks also to ***Aino Salmivaara, Suvi Koskinen, Aino Lipasti, Anna Äyräväinen, Maija Rinta-Aho, Jarno Kettunen and Valtteri Honkanen*** who unselfishly helped me with acquiring samples for my last study.

Isille ja äidille kiitos kaikesta tuesta, rakkaudesta ja välittämästänne innosta tieteelliseen työhön. Te kannustitte minua eteenpäin ja sain teiltä mahdollisuuden opiskella rauhassa ja nyt tehdä työtä, josta pidän. Atelle kiitos siitä että olet paras veli. Näit väitöskirjan hienona asiana. Valtterille kiitos että otit minut elämääsi ja pidit minusta kiinni, ja kiitos suuresta kannustuksesta ja tuesta väitöskirjan kirjoitusvaiheessa.

References

1. Primeau V, Coderre L, Karelis AD, Brochu M, Lavoie ME, Messier V, et al. Characterizing the profile of obese patients who are metabolically healthy. Int J Obes (Lond). 2011 Jul;35(7):971-81.

2. Bluher M. The distinction of metabolically 'healthy' from 'unhealthy' obese individuals. Curr Opin Lipidol. 2010 Feb;21(1):38-43.

3. Bjorntorp P, Gustafson A, Persson B. Adipose tissue fat cell size and number in relation to metabolism in endogenous hypertriglyceridemia. Acta Med Scand. 1971 Nov;190(5):363-7.

4. Pietilainen KH, Naukkarinen J, Rissanen A, Saharinen J, Ellonen P, Keranen H, et al. Global transcript profiles of fat in monozygotic twins discordant for BMI: pathways behind acquired obesity. PLoS Med. 2008 Mar 11;5(3):e51.

5. Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE. Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. Diabetologia. 2000 Dec;43(12):1498-506.

6. De Pauw A, Tejerina S, Raes M, Keijer J, Arnould T. Mitochondrial (dys)function in adipocyte (de)differentiation and systemic metabolic alterations. Am J Pathol. 2009 Sep;175(3):927-39.

7. Goossens GH. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. Physiol Behav. 2008 May 23;94(2):206-18.

8. Rosen ED, Spiegelman BM. What we talk about when we talk about fat. Cell. 2014 Jan 16;156(1-2):20-44.

9. Goossens GH, Blaak EE. Adipose tissue dysfunction and impaired metabolic health in human obesity: a matter of oxygen? Front Endocrinol (Lausanne). 2015;6:55.

10. Yin X, Lanza IR, Swain JM, Sarr MG, Nair KS, Jensen MD. Adipocyte mitochondrial function is reduced in human obesity independent of fat cell size. J Clin Endocrinol Metab. 2014 Feb;99(2):E209-16.

11. Messier V, Karelis AD, Prud'homme D, Primeau V, Brochu M, Rabasa-Lhoret R. Identifying metabolically healthy but obese individuals in sedentary postmenopausal women. Obesity (Silver Spring). 2010 May;18(5):911-7.

12. Weiss R, Taksali SE, Dufour S, Yeckel CW, Papademetris X, Cline G, et al. The "obese insulin-sensitive" adolescent: importance of adiponectin and lipid partitioning. J Clin Endocrinol Metab. 2005 Jun;90(6):3731-7.

13. Brochu M, Tchernof A, Dionne IJ, Sites CK, Eltabbakh GH, Sims EA, et al. What are the physical characteristics associated with a normal metabolic profile despite a high level of obesity in postmenopausal women?. J Clin Endocrinol Metab. 2001 Mar;86(3):1020-5.

14. Kloting N, Fasshauer M, Dietrich A, Kovacs P, Schon MR, Kern M, et al. Insulin-sensitive obesity. Am J Physiol Endocrinol Metab. 2010 Sep;299(3):E506-15.

15. Karelis AD, Faraj M, Bastard J, St-Pierre DH, Brochu M, Prud'homme D, et al. The metabolically healthy but obese individual presents a favorable inflammation profile. J Clin Endocrinol Metab. 2005 Jul;90(7):4145-50.

16. Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K, et al. Identification and characterization of metabolically benign obesity in humans. Arch Intern Med. 2008 Aug 11;168(15):1609-16.

17. Heilbronn L, Smith SR, Ravussin E. Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance and type II diabetes mellitus. Int J Obes Relat Metab Disord. 2004 Dec;28(Suppl 4):S12-21.

18. McQuaid SE, Hodson L, Neville MJ, Dennis AL, Cheeseman J, Humphreys SM, et al. Downregulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition?. Diabetes. 2011 Jan;60(1):47-55.

19. Pietilainen KH, Rog T, Seppanen-Laakso T, Virtue S, Gopalacharyulu P, Tang J, et al. Association of lipidome remodeling in the adipocyte membrane with acquired obesity in humans. PLoS Biol. 2011 Jun;9(6):e1000623.

20. Hojlund K, Mogensen M, Sahlin K, Beck-Nielsen H. Mitochondrial dysfunction in type 2 diabetes and obesity. Endocrinol Metab Clin North Am. 2008 x; Sep;37(3):713-31.

21. Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, et al. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. Lancet. 2011 Feb 12;377(9765):557-67.

22. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity among adults: United States, 2011-2012. NCHS Data Brief. 2013.

23. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA. 2014 Feb 26;311(8):806-14.

24. Berteus Forslund H, Torgerson JS, Sjostrom L, Lindroos AK. Snacking frequency in relation to energy intake and food choices in obese men and women compared to a reference population. Int J Obes (Lond). 2005 Jun;29(6):711-9.

25. van der Klaauw AA, Farooqi IS. The hunger genes: pathways to obesity. Cell. 2015 Mar 26;161(1):119-32.

26. Blundell JE, Gibbons C, Caudwell P, Finlayson G, Hopkins M. Appetite control and energy balance: impact of exercise. Obes Rev. 2015 Feb;16(Suppl 1):67-76.

27. Walley AJ, Blakemore AIF, Froguel P. Genetics of obesity and the prediction of risk for health. Hum Mol Genet. 2006 Oct 15;15(Spec 2):R124-30.

28. Keith SW, Redden DT, Katzmarzyk PT, Boggiano MM, Hanlon EC, Benca RM, et al. Putative contributors to the secular increase in obesity: exploring the roads less traveled. Int J Obes (Lond). 2006 Nov;30(11):1585-94.

29. Cani PD, Delzenne NM. Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. Curr Opin Pharmacol. 2009 Dec;9(6):737-43.

30. Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the United States. JAMA. 2001 Sep 12;286(10):1195-200.

31. Zimmet P, Magliano D, Matsuzawa Y, Alberti G, Shaw J. The metabolic syndrome: a global public health problem and a new definition. J Atheroscler Thromb. 2005;12(6):295-300.

32. Kasuga M. Insulin resistance and pancreatic beta cell failure. J Clin Invest. 2006 Jul;116(7):1756-60.

33. Boden G, Laakso M. Lipids and glucose in type 2 diabetes: what is the cause and effect? Diabetes Care. 2004 Sep;27(9):2253-9.

34. Arcaro G, Zamboni M, Rossi L, Turcato E, Covi G, Armellini F, et al. Body fat distribution predicts the degree of endothelial dysfunction in uncomplicated obesity. Int J Obes Relat Metab Disord. 1999 Sep;23(9):936-42.

35. Westerbacka J, Vehkavaara S, Bergholm R, Wilkinson I, Cockcroft J, Yki-Jarvinen H. Marked resistance of the ability of insulin to decrease arterial stiffness characterizes human obesity. Diabetes. 1999 Apr;48(4):821-7.

36. Heilbronn LK, Campbell LV. Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. Curr Pharm Des. 2008;14(12):1225-30.

37. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. BMC Public Health. 2009;9:88.

38. Shah N, Roux F. The relationship of obesity and obstructive sleep apnea. Clin Chest Med. 2009 vii; Sep;30(3):455-65.

39. Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BWJH, et al. Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies. Arch Gen Psychiatry. 2010 Mar;67(3):220-9.

40. Fock KM, Khoo J. Diet and exercise in management of obesity and overweight. J Gastroenterol Hepatol. 2013 Dec;28(Suppl 4):59-63.

41. Sacks FM, Bray GA, Carey VJ, Smith SR, Ryan DH, Anton SD, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. N Engl J Med. 2009 Feb 26;360(9):859-73.

42. Miller WC, Koceja DM, Hamilton EJ. A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention. Int J Obes Relat Metab Disord. 1997 Oct;21(10):941-7.

43. Slentz CA, Duscha BD, Johnson JL, Ketchum K, Aiken LB, Samsa GP, et al. Effects of the amount of exercise on body weight, body composition, and measures of central obesity: STRRIDE--a randomized controlled study. Arch Intern Med. 2004 Jan 12;164(1):31-9.

44. Kusminski CM, Bickel PB, Scherer PE.
Targeting adipose tissue
in the treatment of
obesity-associated diabetes

. Nat Rev Drug Discov [Epub ahead of print]. 2016 2016 Jun 3.

45. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. Am J Clin Nutr. 2006 Feb;83(2):461S-5S.

46. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. J Clin Invest. 2011 Jun;121(6):2094-101.

47. Ravussin E, Smith SR. Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus. Ann N Y Acad Sci. 2002 Jun;967:363-78.

48. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev. 2000 Dec;21(6):697-738.

49. Marin P, Andersson B, Ottosson M, Olbe L, Chowdhury B, Kvist H, et al. The morphology and metabolism of intraabdominal adipose tissue in men. Metabolism. 1992 Nov;41(11):1242-8.

50. Ross R, Shaw KD, Rissanen J, Martel Y, de Guise J, Avruch L. Sex differences in lean and adipose tissue distribution by magnetic resonance imaging: anthropometric relationships. Am J Clin Nutr. 1994 Jun;59(6):1277-85.

51. Manolopoulos KN, Karpe F, Frayn KN. Gluteofemoral body fat as a determinant of metabolic health. Int J Obes (Lond). 2010 Jun;34(6):949-59.

52. Bouchard C, Despres JP, Mauriege P. Genetic and nongenetic determinants of regional fat distribution. Endocr Rev. 1993 Feb;14(1):72-93.

53. Fruhbeck G. Overview of adipose tissue and its role in obesity and metabolic disorders. Methods Mol Biol. 2008;456:1-22.

54. Arner P. Regional adipocity in man. J Endocrinol. 1997 Nov;155(2):191-2.

55. Hausman DB, DiGirolamo M, Bartness TJ, Hausman GJ, Martin RJ. The biology of white adipocyte proliferation. Obes Rev. 2001 Nov;2(4):239-54.

56. Baker GL. Human adipose tissue composition and age. Am J Clin Nutr. 1969 Jul;22(7):829-35.

57. Curat CA, Miranville A, Sengenes C, Diehl M, Tonus C, Busse R, et al. From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. Diabetes. 2004 May;53(5):1285-92.

58. Sengenes C, Lolmede K, Zakaroff-Girard A, Busse R, Bouloumie A. Preadipocytes in the human subcutaneous adipose tissue display distinct features from the adult mesenchymal and hematopoietic stem cells. J Cell Physiol. 2005 Oct;205(1):114-22.

59. Rodeheffer MS, Birsoy K, Friedman JM. Identification of white adipocyte progenitor cells in vivo. Cell. 2008 Oct 17;135(2):240-9.

60. Cawthorn WP, Scheller EL, MacDougald OA. Adipose tissue stem cells meet preadipocyte commitment: going back to the future. J Lipid Res. 2012 Feb;53(2):227-46.

61. Hirsch J, Gallian E. Methods for the determination of adipose cell size in man and animals. J Lipid Res. 1968 Jan;9(1):110-9.

62. Giordano A, Frontini A, Cinti S. Convertible visceral fat as a therapeutic target to curb obesity. Nat Rev Drug Discov. 2016 Jun;15(6):405-24.

63. Morrison SF, Madden CJ, Tupone D. Central neural regulation of brown adipose tissue thermogenesis and energy expenditure. Cell Metab. 2014 May 6;19(5):741-56.

64. Boudina S, Graham TE. Mitochondrial function/dysfunction in white adipose tissue. Exp Physiol. 2014 Sep;99(9):1168-78.

65. Kusminski CM, Scherer PE. Mitochondrial dysfunction in white adipose tissue. Trends Endocrinol Metab. 2012 Sep;23(9):435-43.

66. Wilson-Fritch L, Burkart A, Bell G, Mendelson K, Leszyk J, Nicoloro S, et al. Mitochondrial biogenesis and remodeling during adipogenesis and in response to the insulin sensitizer rosiglitazone. Mol Cell Biol. 2003 Feb;23(3):1085-94.

67. Rong JX, Qiu Y, Hansen MK, Zhu L, Zhang V, Xie M, et al. Adipose mitochondrial biogenesis is suppressed in db/db and high-fat diet-fed mice and improved by rosiglitazone. Diabetes. 2007 Jul;56(7):1751-60.

68. Yehuda-Shnaidman E, Buehrer B, Pi J, Kumar N, Collins S. Acute stimulation of white adipocyte respiration by PKA-induced lipolysis. Diabetes. 2010 Oct;59(10):2474-83.

69. Goldenthal MJ, Marin-Garcia J. Mitochondrial signaling pathways: a receiver/integrator organelle. Mol Cell Biochem. 2004 Jul;262(1-2):1-16.

70. McKenzie M, Lazarou M, Ryan MT. Chapter 18 Analysis of respiratory chain complex assembly with radiolabeled nuclear- and mitochondrial-encoded subunits. Methods Enzymol. 2009;456:321-39.

71. Falkenberg M, Larsson NG, Gustafsson CM. DNA replication and transcription in mammalian mitochondria. Annu Rev Biochem. 2007;76:679-99.

72. O'Brien TW. Properties of human mitochondrial ribosomes. IUBMB Life. 2003 Sep;55(9):505-13.

73. O'Brien TW. The general occurrence of 55 S ribosomes in mammalian liver mitochondria. J Biol Chem. 1971 May 25;246(10):3409-17.

74. Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. Cell. 2012 Mar 16;148(6):1145-59.

75. Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong SE, et al. A mitochondrial protein compendium elucidates complex I disease biology. Cell. 2008 Jul 11;134(1):112-23.

76. Neupert W, Herrmann JM. Translocation of proteins into mitochondria. Annu Rev Biochem. 2007;76:723-49.

77. Schmidt O, Pfanner N, Meisinger C. Mitochondrial protein import: from proteomics to functional mechanisms. Nat Rev Mol Cell Biol. 2010 Sep;11(9):655-67.

78. Hoogenraad NJ, Ryan MT. Translocation of proteins into mitochondria. IUBMB Life. 2001 Jun;51(6):345-50.

79. Schagger H, Pfeiffer K. Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. EMBO J. 2000 Apr 17;19(8):1777-83.

80. Acin-Perez R, Bayona-Bafaluy MP, Fernandez-Silva P, Moreno-Loshuertos R, Perez-Martos A, Bruno C, et al. Respiratory complex III is required to maintain complex I in mammalian mitochondria. Mol Cell. 2004 Mar 26;13(6):805-15.

81. Schagger H, de Coo R, Bauer MF, Hofmann S, Godinot C, Brandt U. Significance of respirasomes for the assembly/stability of human respiratory chain complex I. J Biol Chem. 2004 Aug 27;279(35):36349-53.

82. Nicholls DG. Mitochondrial ion circuits. Essays Biochem. 2010;47:25-35.

83. Okuno D, Iino R, Noji H. Rotation and structure of FoF1-ATP synthase. J Biochem (Tokyo). 2011 Jun;149(6):655-64.

84. Schon EA, DiMauro S, Hirano M. Human mitochondrial DNA: roles of inherited and somatic mutations. Nat Rev Genet. 2012 Dec;13(12):878-90.

85. Lapuente-Brun E, Moreno-Loshuertos R, Acin-Perez R, Latorre-Pellicer A, Colas C, Balsa E, et al. Supercomplex assembly determines electron flux in the mitochondrial electron transport chain. Science. 2013 Jun 28;340(6140):1567-70.

86. Murphy MP. How mitochondria produce reactive oxygen species. Biochem J. 2009 Jan 1;417(1):1-13.

87. Pitkanen S, Robinson BH. Mitochondrial complex I deficiency leads to increased production of superoxide radicals and induction of superoxide dismutase. J Clin Invest. 1996 Jul 15;98(2):345-51.

88. Carroll J, Fearnley IM, Skehel JM, Shannon RJ, Hirst J, Walker JE. Bovine complex I is a complex of 45 different subunits. J Biol Chem. 2006 Oct 27;281(43):32724-7.

89. Iwata S, Lee JW, Okada K, Lee JK, Iwata M, Rasmussen B, et al. Complete structure of the 11-subunit bovine mitochondrial cytochrome bc1 complex. Science. 1998 Jul 3;281(5373):64-71.

90. Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, et al. The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A. Science. 1996 May 24;272(5265):1136-44.

91. Owen OE, Kalhan SC, Hanson RW. The key role of anaplerosis and cataplerosis for citric acid cycle function. J Biol Chem. 2002 Aug 23;277(34):30409-12.

92. Bartlett K, Eaton S. Mitochondrial beta-oxidation. Eur J Biochem. 2004 Feb;271(3):462-9.

93. Stocco DM. The role of the StAR protein in steroidogenesis: challenges for the future. J Endocrinol. 2000 Mar;164(3):247-53.

94. Rouault TA, Tong W. Iron-sulphur cluster biogenesis and mitochondrial iron homeostasis. Nat Rev Mol Cell Biol. 2005 Apr;6(4):345-51.

95. Ajioka RS, Phillips JD, Kushner JP. Biosynthesis of heme in mammals. Biochim Biophys Acta. 2006 Jul;1763(7):723-36.

96. Parsons MJ, Green DR. Mitochondria in cell death. Essays Biochem. 2010;47:99-114.

97. Gunter TE, Yule DI, Gunter KK, Eliseev RA, Salter JD. Calcium and mitochondria. FEBS Lett. 2004 Jun 1;567(1):96-102.

98. Detmer SA, Chan DC. Functions and dysfunctions of mitochondrial dynamics. Nat Rev Mol Cell Biol. 2007 Nov;8(11):870-9.

99. Frazier AE, Kiu C, Stojanovski D, Hoogenraad NJ, Ryan MT. Mitochondrial morphology and distribution in mammalian cells. Biol Chem. 2006 Dec;387(12):1551-8.

100. Gomes LC, Di Benedetto G, Scorrano L. During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. Nat Cell Biol. 2011 May;13(5):589-98.

101. Molina AJA, Wikstrom JD, Stiles L, Las G, Mohamed H, Elorza A, et al. Mitochondrial networking protects beta-cells from nutrient-induced apoptosis. Diabetes. 2009 Oct;58(10):2303-15.

102. Hoppeler H, Fluck M. Plasticity of skeletal muscle mitochondria: structure and function. Med Sci Sports Exerc. 2003 Jan;35(1):95-104.

103. Bogacka I, Ukropcova B, McNeil M, Gimble JM, Smith SR. Structural and functional consequences of mitochondrial biogenesis in human adipocytes in vitro. J Clin Endocrinol Metab. 2005 Dec;90(12):6650-6.

104. Choo H, Kim J, Kwon O, Lee CS, Mun JY, Han SS, et al. Mitochondria are impaired in the adipocytes of type 2 diabetic mice. Diabetologia. 2006 Apr;49(4):784-91.

105. Sutherland LN, Capozzi LC, Turchinsky NJ, Bell RC, Wright DC. Time course of high-fat diet-induced reductions in adipose tissue mitochondrial proteins: potential mechanisms and the relationship to glucose intolerance. Am J Physiol Endocrinol Metab. 2008 Nov;295(5):E1076-83.

106. Hardie DG. AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function. Genes Dev. 2011 Sep 15;25(18):1895-908.

107. Mihaylova MM, Shaw RJ. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. Nat Cell Biol. 2011 Sep;13(9):1016-23.

108. Carling D, Mayer FV, Sanders MJ, Gamblin SJ. AMP-activated protein kinase: nature's energy sensor. Nat Chem Biol. 2011 Aug;7(8):512-8.

109. Gao AW, Canto C, Houtkooper RH. Mitochondrial response to nutrient availability and its role in metabolic disease. EMBO Mol Med. 2014 May;6(5):580-9.

110. Andreux PA, Houtkooper RH, Auwerx J. Pharmacological approaches to restore mitochondrial function. Nat Rev Drug Discov. 2013 Jun;12(6):465-83.

111. Handschin C, Spiegelman BM. Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. Endocr Rev. 2006 Dec;27(7):728-35.

112. Scarpulla RC. Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. Biochim Biophys Acta. 2011 Jul;1813(7):1269-78.

113. Houtkooper RH, Canto C, Wanders RJ, Auwerx J. The secret life of NAD+: an old metabolite controlling new metabolic signaling pathways. Endocr Rev. 2010 Apr;31(2):194-223.

114. Virbasius JV, Scarpulla RC. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. Proc Natl Acad Sci U S A. 1994 Feb 15;91(4):1309-13.

115. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell. 1999 Jul 9;98(1):115-24.

116. Schreiber SN, Emter R, Hock MB, Knutti D, Cardenas J, Podvinec M, et al. The estrogen-related receptor alpha (ERRalpha) functions in PPARgamma coactivator 1alpha (PGC-1alpha)-induced mitochondrial biogenesis. Proc Natl Acad Sci U S A. 2004 Apr 27;101(17):6472-7.

117. Mootha VK, Handschin C, Arlow D, Xie X, St Pierre J, Sihag S, et al. Erralpha and Gabpa/b specify PGC-1alpha-dependent oxidative phosphorylation gene expression that is altered in diabetic muscle. Proc Natl Acad Sci U S A. 2004 Apr 27;101(17):6570-5.

118. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science. 2004 Mar 26;303(5666):2011-5.

119. Gray SL, Vidal-Puig AJ. Adipose tissue expandability in the maintenance of metabolic homeostasis. Nutr Rev. 2007 Jun;65(6 Pt 2):S7-12.

120. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. Nature. 2008 Jun 5;453(7196):783-7.

121. Kras KM, Hausman DB, Hausman GJ, Martin RJ. Adipocyte development is dependent upon stem cell recruitment and proliferation of preadipocytes. Obes Res. 1999 Sep;7(5):491-7.

122. Guilak F, Lott KE, Awad HA, Cao Q, Hicok KC, Fermor B, et al. Clonal analysis of the differentiation potential of human adipose-derived adult stem cells. J Cell Physiol. 2006 Jan;206(1):229-37.

123. Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, et al. White fat progenitor cells reside in the adipose vasculature. Science. 2008 Oct 24;322(5901):583-6.

124. Medici D, Shore EM, Lounev VY, Kaplan FS, Kalluri R, Olsen BR. Conversion of vascular endothelial cells into multipotent stem-like cells. Nat Med. 2010 Dec;16(12):1400-6.

125. Monteiro MC, Wdziekonski B, Villageois P, Vernochet C, Iehle C, Billon N, et al. Commitment of mouse embryonic stem cells to the adipocyte lineage requires retinoic acid receptor beta and active GSK3. Stem Cells Dev. 2009 Apr;18(3):457-63.

126. Crossno JTJ, Majka SM, Grazia T, Gill RG, Klemm DJ. Rosiglitazone promotes development of a novel adipocyte population from bone marrow-derived circulating progenitor cells. J Clin Invest. 2006 Dec;116(12):3220-8.

127. Takashima Y, Era T, Nakao K, Kondo S, Kasuga M, Smith AG, et al. Neuroepithelial cells supply an initial transient wave of MSC differentiation. Cell. 2007 Jun 29;129(7):1377-88.

128. Lefterova MI, Lazar MA. New developments in adipogenesis. Trends Endocrinol Metab. 2009 Apr;20(3):107-14.

129. Lowe CE, O'Rahilly S, Rochford JJ. Adipogenesis at a glance. J Cell Sci. 2011 Aug 15;124(Pt 16):2681-6.

130. Prestwich TC, Macdougald OA. Wnt/beta-catenin signaling in adipogenesis and metabolism. Curr Opin Cell Biol. 2007 Dec;19(6):612-7.

131. Cousin W, Fontaine C, Dani C, Peraldi P. Hedgehog and adipogenesis: fat and fiction. Biochimie. 2007 Dec;89(12):1447-53.

132. Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. Physiol Rev. 1998 Jul;78(3):783-809.

133. Rosen ED, Spiegelman BM. PPARgamma : a nuclear regulator of metabolism, differentiation, and cell growth. J Biol Chem. 2001 Oct 12;276(41):37731-4.

134. Hamm JK, el Jack AK, Pilch PF, Farmer SR. Role of PPAR gamma in regulating adipocyte differentiation and insulin-responsive glucose uptake. Ann N Y Acad Sci. 1999 Nov 18;892:134-45.

135. Kim JB, Spiegelman BM. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev. 1996 May 1;10(9):1096-107.

136. Laplante M, Sabatini DM. mTOR signaling at a glance. J Cell Sci. 2009 Oct 15;122(Pt 20):3589-94.

137. Nakae J, Kitamura T, Kitamura Y, Biggs WH3, Arden KC, Accili D. The forkhead transcription factor Foxo1 regulates adipocyte differentiation. Dev Cell. 2003 Jan;4(1):119-29.

138. Kim JE, Chen J. regulation of peroxisome proliferator-activated receptor-gamma activity by mammalian target of rapamycin and amino acids in adipogenesis. Diabetes. 2004 Nov;53(11):2748-56.

139. Nugent C, Prins JB, Whitehead JP, Savage D, Wentworth JM, Chatterjee VK, et al. Potentiation of glucose uptake in 3T3-L1 adipocytes by PPAR gamma agonists is maintained in cells expressing a PPAR gamma dominant-negative mutant: evidence for selectivity in the downstream responses to PPAR gamma activation. Mol Endocrinol. 2001 Oct;15(10):1729-38.

140. SHELDON H, HOLLENBERG CH, WINEGRAD AI. Observations on the morphology of adipose tissue. I. The fine structure of cells from fasted and diabetic rats. Diabetes. 1962 Sep-Oct;11:378-87.

141. Novikoff AB, Novikoff PM, Rosen OM, Rubin CS. Organelle relationships in cultured 3T3-L1 preadipocytes. J Cell Biol. 1980 Oct;87(1):180-96.

142. Wilson-Fritch L, Nicoloro S, Chouinard M, Lazar MA, Chui PC, Leszyk J, et al. Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. J Clin Invest. 2004 Nov;114(9):1281-9.

143. Leonardsson G, Steel JH, Christian M, Pocock V, Milligan S, Bell J, et al. Nuclear receptor corepressor RIP140 regulates fat accumulation. Proc Natl Acad Sci U S A. 2004 Jun 1;101(22):8437-42.

144. Ijichi N, Ikeda K, Horie-Inoue K, Yagi K, Okazaki Y, Inoue S. Estrogen-related receptor alpha modulates the expression of adipogenesis-related genes during adipocyte differentiation. Biochem Biophys Res Commun. 2007 Jul 6;358(3):813-8.

145. Spiegelman BM, Puigserver P, Wu Z. Regulation of adipogenesis and energy balance by PPARgamma and PGC-1. Int J Obes Relat Metab Disord. 2000 Nov;24(Suppl 4):S8-10.

146. Carriere A, Fernandez Y, Rigoulet M, Penicaud L, Casteilla L. Inhibition of preadipocyte proliferation by mitochondrial reactive oxygen species. FEBS Lett. 2003 Aug 28;550(1-3):163-7.

147. Carriere A, Carmona M, Fernandez Y, Rigoulet M, Wenger RH, Penicaud L, et al. Mitochondrial reactive oxygen species control the transcription factor CHOP-10/GADD153 and adipocyte differentiation: a mechanism for hypoxia-dependent effect. J Biol Chem. 2004 Sep 24;279(39):40462-9.

148. Tormos KV, Anso E, Hamanaka RB, Eisenbart J, Joseph J, Kalyanaraman B, et al. Mitochondrial complex III ROS regulate adipocyte differentiation. Cell Metab. 2011 Oct 5;14(4):537-44.

149. Kersten S. Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Rep. 2001 Apr;2(4):282-6.

150. Kazantzis M, Stahl A. Fatty acid transport proteins, implications in physiology and disease. Biochim Biophys Acta. 2012 May;1821(5):852-7.

151. Rognstad R, Katz J. The effect of 2,4-dinitrophenol on adipose-tissue metabolism. Biochem J. 1969 Feb;111(4):431-44.

152. Kopecky J, Rossmeisl M, Flachs P, Bardova K, Brauner P. Mitochondrial uncoupling and lipid metabolism in adipocytes. Biochem Soc Trans. 2001 Nov;29(Pt 6):791-7.

153. Bremer J, Bjerve KS, Borrebaek B, Christiansen R. The glycerophosphateacyltransferases and their function in the metabolism of fatty acids. Mol Cell Biochem. 1976 Aug 30;12(2):113-25.

154. Clifford GM, Londos C, Kraemer FB, Vernon RG, Yeaman SJ. Translocation of hormone-sensitive lipase and perilipin upon lipolytic stimulation of rat adipocytes. J Biol Chem. 2000 Feb 18;275(7):5011-5.

155. Sztalryd C, Kraemer FB. Regulation of hormone-sensitive lipase in streptozotocin-induced diabetic rats. Metabolism. 1995 Nov;44(11):1391-6.

156. Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science. 2004 Nov 19;306(5700):1383-6.

157. Fassina G, Dorigo P, Gaion RM. Equilibrium between metabolic pathways producing energy: a key factor in regulating lipolysis. Pharmacol Res Commun. 1974 Feb;6(1):1-21.

158. Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS. Regulation of lipolysis in adipocytes. Annu Rev Nutr. 2007;27:79-101.

159. Shrago E, Spennetta T, Gordon E. Fatty acid synthesis in human adipose tissue. J Biol Chem. 1969 May 25;244(10):2761-6.

160. Ballard FJ, Hanson RW, Leveille GA. Phosphoenolpyruvate carboxykinase and the synthesis of glyceride-glycerol from pyruvate in adipose tissue. J Biol Chem. 1967 Jun 10;242(11):2746-50.

161. Reshef L, Olswang Y, Cassuto H, Blum B, Croniger CM, Kalhan SC, et al. Glyceroneogenesis and the triglyceride/fatty acid cycle. J Biol Chem. 2003 Aug 15;278(33):30413-6.

162. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem. 1997 Feb 15;244(1):1-14.

163. Yeh LA, Lee KH, Kim KH. Regulation of rat liver acetyl-CoA carboxylase. Regulation of phosphorylation and inactivation of acetyl-CoA carboxylase by the adenylate energy charge. J Biol Chem. 1980 Mar 25;255(6):2308-14.

164. Rosenthal J, Angel A, Farkas J. Metabolic fate of leucine: a significant sterol precursor in adipose tissue and muscle. Am J Physiol. 1974 Feb;226(2):411-8.

165. Herman MA, She P, Peroni OD, Lynch CJ, Kahn BB. Adipose tissue branched chain amino acid (BCAA) metabolism modulates circulating BCAA levels. J Biol Chem. 2010 Apr 9;285(15):11348-56.

166. She P, Van Horn C, Reid T, Hutson SM, Cooney RN, Lynch CJ. Obesity-related elevations in plasma leucine are associated with alterations in enzymes involved in branched-chain amino acid metabolism. Am J Physiol Endocrinol Metab. 2007 Dec;293(6):E1552-63.

167. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol. 2014 Dec;10(12):723-36.

168. Felig P, Marliss E, Cahill GFJ. Plasma amino acid levels and insulin secretion in obesity. N Engl J Med. 1969 Oct 9;281(15):811-6.

169. Tischler ME, Goldberg AL. Leucine degradation and release of glutamine and alanine by adipose tissue. J Biol Chem. 1980 Sep 10;255(17):8074-81.

170. Suryawan A, Hawes JW, Harris RA, Shimomura Y, Jenkins AE, Hutson SM. A molecular model of human branched-chain amino acid metabolism. Am J Clin Nutr. 1998 Jul;68(1):72-81.

171. Hutson SM, Wallin R, Hall TR. Identification of mitochondrial branched chain aminotransferase and its isoforms in rat tissues. J Biol Chem. 1992 Aug 5;267(22):15681-6.

172. Kitsy A, Carney S, Vivar JC, Knight MS, Pointer MA, Gwathmey JK, et al. Effects of leucine supplementation and serum withdrawal on branched-chain amino acid pathway gene and protein expression in mouse adipocytes. PLoS ONE. 2014;9(7):e102615.

173. Lackey DE, Lynch CJ, Olson KC, Mostaedi R, Ali M, Smith WH, et al. Regulation of adipose branched-chain amino acid catabolism enzyme expression and cross-adipose amino acid flux in human obesity. Am J Physiol Endocrinol Metab. 2013 Jun 1;304(11):E1175-87.

174. Kahn BB, Cushman SW. Subcellular translocation of glucose transporters: role in insulin action and its perturbation in altered metabolic states. Diabetes Metab Rev. 1985;1(3):203-27.

175. Frayn KN, Humphreys SM. Metabolic characteristics of human subcutaneous abdominal adipose tissue after overnight fast. Am J Physiol Endocrinol Metab. 2012 Feb 15;302(4):E468-75.

176. Guan H, Li Y, Jensen MV, Newgard CB, Steppan CM, Lazar MA. A futile metabolic cycle activated in adipocytes by antidiabetic agents. Nat Med. 2002 Oct;8(10):1122-8.

177. Forest C, Tordjman J, Glorian M, Duplus E, Chauvet G, Quette J, et al. Fatty acid recycling in adipocytes: a role for glyceroneogenesis and phosphoenolpyruvate carboxykinase. Biochem Soc Trans. 2003 Dec;31(Pt 6):1125-9.

178. Bluher M, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, et al. Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. Dev Cell. 2002 Jul;3(1):25-38.

179. Rasouli N, Kern PA. Adipocytokines and the metabolic complications of obesity. J Clin Endocrinol Metab. 2008 Nov;93(11 Suppl 1):S64-73.

180. Ahima RS. Adipose tissue as an endocrine organ. Obesity (Silver Spring). 2006 Aug;14(Suppl 5):242S-9S.

181. Mohamed-Ali V, Pinkney JH, Coppack SW. Adipose tissue as an endocrine and paracrine organ. Int J Obes Relat Metab Disord. 1998 Dec;22(12):1145-58.

182. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med. 2006 Feb 9;354(6):610-21.

183. Frayn KN, Karpe F, Fielding BA, Macdonald IA, Coppack SW. Integrative physiology of human adipose tissue. Int J Obes Relat Metab Disord. 2003 Aug;27(8):875-88.

184. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004 Jun;89(6):2548-56.

185. Fruhbeck G, Gomez-Ambrosi J, Muruzabal FJ, Burrell MA. The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. Am J Physiol Endocrinol Metab. 2001 Jun;280(6):E827-47.

186. Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab. 2007 Mar;92(3):1023-33.

187. Otero M, Lago R, Lago F, Casanueva FF, Dieguez C, Gomez-Reino JJ, et al. Leptin, from fat to inflammation: old questions and new insights. FEBS Lett. 2005 Jan 17;579(2):295-301.

188. Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, et al. Role of leptin in the neuroendocrine response to fasting. Nature. 1996 Jul 18;382(6588):250-2.

189. Bjorbaek C, Kahn BB. Leptin signaling in the central nervous system and the periphery. Recent Prog Horm Res. 2004;59:305-31.

190. Takekoshi K, Motooka M, Isobe K, Nomura F, Manmoku T, Ishii K, et al. Leptin directly stimulates catecholamine secretion and synthesis in cultured porcine adrenal medullary chromaffin cells. Biochem Biophys Res Commun. 1999 Aug 2;261(2):426-31.

191. Gainsford T, Willson TA, Metcalf D, Handman E, McFarlane C, Ng A, et al. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. Proc Natl Acad Sci U S A. 1996 Dec 10;93(25):14564-8.

192. Gan L, Guo K, Cremona ML, McGraw TE, Leibel RL, Zhang Y. TNF-alpha up-regulates protein level and cell surface expression of the leptin receptor by stimulating its export via a PKC-dependent mechanism. Endocrinology. 2012 Dec;153(12):5821-33.

193. Kadowaki T, Yamauchi T, Kubota N. The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. FEBS Lett. 2008 Jan 9;582(1):74-80.

194. Heilbronn LK, Smith SR, Ravussin E. The insulin-sensitizing role of the fat derived hormone adiponectin. Curr Pharm Des. 2003;9(17):1411-8.

195. Wedellova Z, Dietrich J, Siklova-Vitkova M, Kolostova K, Kovacikova M, Duskova M, et al. Adiponectin inhibits spontaneous and catecholamine-induced lipolysis in human adipocytes of non-obese subjects through AMPK-dependent mechanisms. Physiol Res. 2011;60(1):139-48.

196. Lazra Y, Falach A, Frenkel L, Rozenberg K, Sampson S, Rosenzweig T. Autocrine/paracrine function of globular adiponectin: inhibition of lipid metabolism and inflammatory response in 3T3-L1 adipocytes. J Cell Biochem. 2015 May;116(5):754-66.

197. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes. 2001 Sep;50(9):2094-9.

198. Koh EH, Park J, Park H, Jeon MJ, Ryu JW, Kim M, et al. Essential role of mitochondrial function in adiponectin synthesis in adipocytes. Diabetes. 2007 Dec;56(12):2973-81.

199. Qiao L, Kinney B, Yoo HS, Lee B, Schaack J, Shao J. Adiponectin increases skeletal muscle mitochondrial biogenesis by suppressing mitogen-activated protein kinase phosphatase-1. Diabetes. 2012 Jun;61(6):1463-70.

200. Iwabu M, Yamauchi T, Okada-Iwabu M, Sato K, Nakagawa T, Funata M, et al. Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2+) and AMPK/SIRT1. Nature. 2010 Apr 29;464(7293):1313-9.

201. Holland WL, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, et al. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. Nat Med. 2011 Jan;17(1):55-63.

202. Holland WL, Summers SA. Sphingolipids, insulin resistance, and metabolic disease: new insights from in vivo manipulation of sphingolipid metabolism. Endocr Rev. 2008 Jun;29(4):381-402.

203. Salans LB, Knittle JL, Hirsch J. The role of adipose cell size and adipose tissue insulin sensitivity in the carbohydrate intolerance of human obesity. J Clin Invest. 1968 Jan;47(1):153-65.

204. Hoffstedt J, Arner E, Wahrenberg H, Andersson DP, Qvisth V, Lofgren P, et al. Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity. Diabetologia. 2010 Dec;53(12):2496-503.

205. Andersson DP, Eriksson Hogling D, Thorell A, Toft E, Qvisth V, Naslund E, et al. Changes in subcutaneous fat cell volume and insulin sensitivity after weight loss. Diabetes Care. 2014 Jul;37(7):1831-6.

206. Larson-Meyer DE, Heilbronn LK, Redman LM, Newcomer BR, Frisard MI, Anton S, et al. Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. Diabetes Care. 2006 Jun;29(6):1337-44.

207. Lonn M, Mehlig K, Bengtsson C, Lissner L. Adipocyte size predicts incidence of type 2 diabetes in women. FASEB J. 2010 Jan;24(1):326-31.

208. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res. 2005 Nov;46(11):2347-55.

209. Strissel KJ, Stancheva Z, Miyoshi H, Perfield JW2, DeFuria J, Jick Z, et al. Adipocyte death, adipose tissue remodeling, and obesity complications. Diabetes. 2007 Dec;56(12):2910-8.

210. Medina-Gomez G, Virtue S, Lelliott C, Boiani R, Campbell M, Christodoulides C, et al. The link between nutritional status and insulin sensitivity is dependent on the adipocyte-specific peroxisome proliferator-activated receptor-gamma2 isoform. Diabetes. 2005 Jun;54(6):1706-16.

211. Lu Q, Li M, Zou Y, Cao T. Induction of adipocyte hyperplasia in subcutaneous fat depot alleviated type 2 diabetes symptoms in obese mice. Obesity (Silver Spring). 2014 Jul;22(7):1623-31.

212. Holm C, Osterlund T, Laurell H, Contreras JA. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. Annu Rev Nutr. 2000;20:365-93.

213. Pasarica M, Xie H, Hymel D, Bray G, Greenway F, Ravussin E, et al. Lower total adipocyte number but no evidence for small adipocyte depletion in patients with type 2 diabetes. Diabetes Care. 2009 May;32(5):900-2.

214. Drolet R, Richard C, Sniderman AD, Mailloux J, Fortier M, Huot C, et al. Hypertrophy and hyperplasia of abdominal adipose tissues in women. Int J Obes (Lond). 2008 Feb;32(2):283-91.

215. Ryden M, Andersson DP, Bergstrom IB, Arner P. Adipose tissue and metabolic alterations: regional differences in fat cell size and number matter, but differently: a cross-sectional study. J Clin Endocrinol Metab. 2014 Oct;99(10):E1870-6.

216. Kissebah AH, Vydelingum N, Murray R, Evans DJ, Hartz AJ, Kalkhoff RK, et al. Relation of body fat distribution to metabolic complications of obesity. J Clin Endocrinol Metab. 1982 Feb;54(2):254-60.

217. Tran TT, Yamamoto Y, Gesta S, Kahn CR. Beneficial effects of subcutaneous fat transplantation on metabolism. Cell Metab. 2008 May;7(5):410-20.

218. Gesta S, Tseng Y, Kahn CR. Developmental origin of fat: tracking obesity to its source. Cell. 2007 Oct 19;131(2):242-56.

219. Joe AWB, Yi L, Even Y, Vogl AW, Rossi FMV. Depot-specific differences in adipogenic progenitor abundance and proliferative response to high-fat diet. Stem Cells. 2009 Oct;27(10):2563-70.

220. Macotela Y, Emanuelli B, Mori MA, Gesta S, Schulz TJ, Tseng Y, et al. Intrinsic differences in adipocyte precursor cells from different white fat depots. Diabetes. 2012 Jul;61(7):1691-9.

221. Tchoukalova YD, Koutsari C, Votruba SB, Tchkonia T, Giorgadze N, Thomou T, et al. Sex- and depot-dependent differences in adipogenesis in normal-weight humans. Obesity (Silver Spring). 2010 Oct;18(10):1875-80.

222. Tchoukalova YD, Votruba SB, Tchkonia T, Giorgadze N, Kirkland JL, Jensen MD. Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. Proc Natl Acad Sci U S A. 2010 Oct 19;107(42):18226-31.

223. Blaak E. Gender differences in fat metabolism. Curr Opin Clin Nutr Metab Care. 2001 Nov;4(6):499-502.

224. O'Connell J, Lynch L, Cawood TJ, Kwasnik A, Nolan N, Geoghegan J, et al. The relationship of omental and subcutaneous adipocyte size to metabolic disease in severe obesity. PLoS ONE. 2010;5(4):e9997.

225. Rutkowski JM, Stern JH, Scherer PE. The cell biology of fat expansion. J Cell Biol. 2015 Mar 2;208(5):501-12.

226. Gustafson B, Hedjazifar S, Gogg S, Hammarstedt A, Smith U. Insulin resistance and impaired adipogenesis. Trends Endocrinol Metab. 2015 Apr;26(4):193-200.

227. McLaughlin T, Sherman A, Tsao P, Gonzalez O, Yee G, Lamendola C, et al. Enhanced proportion of small adipose cells in insulin-resistant vs insulin-sensitive obese individuals implicates impaired adipogenesis. Diabetologia. 2007 Aug;50(8):1707-15.

228. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AWJ. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003 Dec;112(12):1796-808.

229. Gornicka A, Fettig J, Eguchi A, Berk MP, Thapaliya S, Dixon LJ, et al. Adipocyte hypertrophy is associated with lysosomal permeability both in vivo and in vitro: role in adipose tissue inflammation. Am J Physiol Endocrinol Metab. 2012 Sep 1;303(5):E597-606.

230. Morin CL, Gayles EC, Podolin DA, Wei Y, Xu M, Pagliassotti MJ. Adipose tissue-derived tumor necrosis factor activity correlates with fat cell size but not insulin action in aging rats. Endocrinology. 1998 Dec;139(12):4998-5005.

231. Jernas M, Palming J, Sjoholm K, Jennische E, Svensson PA, Gabrielsson BG, et al. Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. FASEB J. 2006 Jul;20(9):1540-2.

232. Bluher M. Adipose tissue dysfunction contributes to obesity related metabolic diseases. Baillieres Best Pract Res Clin Endocrinol Metab. 2013 Apr;27(2):163-77.

233. Suganami T, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, et al. Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. Arterioscler Thromb Vasc Biol. 2007 Jan;27(1):84-91.

234. Alkhouri N, Gornicka A, Berk MP, Thapaliya S, Dixon LJ, Kashyap S, et al. Adipocyte apoptosis, a link between obesity, insulin resistance, and hepatic steatosis. J Biol Chem. 2010 Jan 29;285(5):3428-38.

235. Fischer B, Schottl T, Schempp C, Fromme T, Hauner H, Klingenspor M, et al. Inverse relationship between body mass index and mitochondrial oxidative phosphorylation capacity in human subcutaneous adipocytes. Am J Physiol Endocrinol Metab. 2015 Aug 15;309(4):E380-7.

236. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature. 2001 Dec 13;414(6865):782-7.

237. Hajer GR, van Haeften TW, Visseren FLJ. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J. 2008 Dec;29(24):2959-71.

238. Kusminski CM, Holland WL, Sun K, Park J, Spurgin SB, Lin Y, et al. MitoNEET-driven alterations in adipocyte mitochondrial activity reveal a crucial adaptive process that preserves insulin sensitivity in obesity. Nat Med. 2012 Oct;18(10):1539-49.

239. Hummasti S, Hotamisligil GS. Endoplasmic reticulum stress and inflammation in obesity and diabetes. Circ Res. 2010 Sep 3;107(5):579-91.

240. Trayhurn P. Hypoxia and adipose tissue function and dysfunction in obesity. Physiol Rev. 2013 Jan;93(1):1-21.

241. Wood IS, de Heredia FP, Wang B, Trayhurn P. Cellular hypoxia and adipose tissue dysfunction in obesity. Proc Nutr Soc. 2009 Nov;68(4):370-7.

242. Chattopadhyay M, Khemka VK, Chatterjee G, Ganguly A, Mukhopadhyay S, Chakrabarti S. Enhanced ROS production and oxidative damage in subcutaneous white adipose tissue mitochondria in obese and type 2 diabetes subjects. Mol Cell Biochem. 2015 Jan;399(1-2):95-103.

243. Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, et al. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab. 2008 Jan;7(1):45-56.

244. Muoio DM, Neufer PD. Lipid-induced mitochondrial stress and insulin action in muscle. Cell Metab. 2012 May 2;15(5):595-605.

245. Cummins TD, Holden CR, Sansbury BE, Gibb AA, Shah J, Zafar N, et al. Metabolic remodeling of white adipose tissue in obesity. Am J Physiol Endocrinol Metab. 2014 Aug 1;307(3):E262-77.

246. Shi X, Burkart A, Nicoloro SM, Czech MP, Straubhaar J, Corvera S. Paradoxical effect of mitochondrial respiratory chain impairment on insulin signaling and glucose transport in adipose cells. J Biol Chem. 2008 Nov 7;283(45):30658-67.

247. Schottl T, Kappler L, Fromme T, Klingenspor M. Limited OXPHOS capacity in white adipocytes is a hallmark of obesity in laboratory mice irrespective of the glucose tolerance status. Mol Metab. 2015 Sep;4(9):631-42.

248. Galgani JE, Moro C, Ravussin E. Metabolic flexibility and insulin resistance. Am J Physiol Endocrinol Metab. 2008 Nov;295(5):E1009-17.

249. Chattopadhyay M, Guhathakurta I, Behera P, Ranjan KR, Khanna M, Mukhopadhyay S, et al. Mitochondrial bioenergetics is not impaired in nonobese subjects with type 2 diabetes mellitus. Metabolism. 2011 Dec;60(12):1702-10.

250. Flachs P, Horakova O, Brauner P, Rossmeisl M, Pecina P, Franssen-van Hal N, et al. Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce beta-oxidation in white fat. Diabetologia. 2005 Nov;48(11):2365-75.

251. Kaaman M, Sparks LM, van Harmelen V, Smith SR, Sjolin E, Dahlman I, et al. Strong association between mitochondrial DNA copy number and lipogenesis in human white adipose tissue. Diabetologia. 2007 Dec;50(12):2526-33.

252. Medina-Gomez G. Mitochondria and endocrine function of adipose tissue. Baillieres Best Pract Res Clin Endocrinol Metab. 2012 Dec;26(6):791-804.

253. Valerio A, Cardile A, Cozzi V, Bracale R, Tedesco L, Pisconti A, et al. TNF-alpha downregulates eNOS expression and mitochondrial biogenesis in fat and muscle of obese rodents. J Clin Invest. 2006 Oct;116(10):2791-8.

254. Wang P, Kuo H, Huang H, Chang AYW, Weng S, Tai M, et al. Biphasic response of mitochondrial biogenesis to oxidative stress in visceral fat of diet-induced obesity mice. Antioxid Redox Signal. 2014 Jun 1;20(16):2572-88.

255. Dahlman I, Forsgren M, Sjogren A, Nordstrom EA, Kaaman M, Naslund E, et al. Downregulation of electron transport chain genes in visceral adipose tissue in type 2 diabetes independent of obesity and possibly involving tumor necrosis factor-alpha. Diabetes. 2006 Jun;55(6):1792-9.

256. Gao C, Zhu C, Zhao Y, Chen X, Ji C, Zhang C, et al. Mitochondrial dysfunction is induced by high levels of glucose and free fatty acids in 3T3-L1 adipocytes. Mol Cell Endocrinol. 2010 May 14;320(1-2):25-33.

257. Vernochet C, Mourier A, Bezy O, Macotela Y, Boucher J, Rardin MJ, et al. Adipose-specific deletion of TFAM increases mitochondrial oxidation and protects mice against obesity and insulin resistance. Cell Metab. 2012 Dec 5;16(6):765-76.

258. Auguet T, Guiu-Jurado E, Berlanga A, Terra X, Martinez S, Porras JA, et al. Downregulation of lipogenesis and fatty acid oxidation in the subcutaneous adipose tissue of morbidly obese women. Obesity (Silver Spring). 2014 Sep;22(9):2032-8.

259. Gao X, Li K, Hui X, Kong X, Sweeney G, Wang Y, et al. Carnitine palmitoyltransferase 1A prevents fatty acid-induced adipocyte dysfunction through suppression of c-Jun N-terminal kinase. Biochem J. 2011 May 1;435(3):723-32.

260. Willson TM, Cobb JE, Cowan DJ, Wiethe RW, Correa ID, Prakash SR, et al. The structure-activity relationship between peroxisome proliferator-activated receptor gamma agonism and the antihyperglycemic activity of thiazolidinediones. J Med Chem. 1996 Feb 2;39(3):665-8.

261. Feinstein DL, Spagnolo A, Akar C, Weinberg G, Murphy P, Gavrilyuk V, et al. Receptor-independent actions of PPAR thiazolidinedione agonists: is mitochondrial function the key? Biochem Pharmacol. 2005 Jul 15;70(2):177-88.

262. Pietilainen KH, Naukkarinen J, Rissanen A, Saharinen J, Ellonen P, Keranen H, et al. Global transcript profiles of fat in monozygotic twins discordant for BMI: pathways behind acquired obesity. PLoS Med. 2008 Mar 11;5(3):e51.

263. Lu J, Xie G, Jia W, Jia W. Insulin resistance and the metabolism of branched-chain amino acids. Front med. 2013 Mar;7(1):53-9.

264. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al. Metabolite profiles and the risk of developing diabetes. Nat Med. 2011 Apr;17(4):448-53.

265. Batch BC, Shah SH, Newgard CB, Turer CB, Haynes C, Bain JR, et al. Branched chain amino acids are novel biomarkers for discrimination of metabolic wellness. Metabolism. 2013 Jul;62(7):961-9.

266. Huang Y, Zhou M, Sun H, Wang Y. Branched-chain amino acid metabolism in heart disease: an epiphenomenon or a real culprit? Cardiovasc Res. 2011 May 1;90(2):220-3.

267. Zhang Y, Guo K, LeBlanc RE, Loh D, Schwartz GJ, Yu Y. Increasing dietary leucine intake reduces diet-induced obesity and improves glucose and cholesterol metabolism in mice via multimechanisms. Diabetes. 2007 Jun;56(6):1647-54.

268. She P, Reid TM, Bronson SK, Vary TC, Hajnal A, Lynch CJ, et al. Disruption of BCATm in mice leads to increased energy expenditure associated with the activation of a futile protein turnover cycle. Cell Metab. 2007 Sep;6(3):181-94.

269. Brahimi-Horn MC, Pouyssegur J. Oxygen, a source of life and stress. FEBS Lett. 2007 Jul 31;581(19):3582-91.

270. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr. 2004 Sep;92(3):347-55.

271. O'Rourke RW, White AE, Metcalf MD, Olivas AS, Mitra P, Larison WG, et al. Hypoxia-induced inflammatory cytokine secretion in human adipose tissue stromovascular cells. Diabetologia. 2011 Jun;54(6):1480-90.

272. Goossens GH, Bizzarri A, Venteclef N, Essers Y, Cleutjens JP, Konings E, et al. Increased adipose tissue oxygen tension in obese compared with lean men is accompanied by insulin resistance, impaired adipose tissue capillarization, and inflammation. Circulation. 2011 Jul 5;124(1):67-76.

273. Virtanen KA, Lonnroth P, Parkkola R, Peltoniemi P, Asola M, Viljanen T, et al. Glucose uptake and perfusion in subcutaneous and visceral adipose tissue during insulin stimulation in nonobese and obese humans. J Clin Endocrinol Metab. 2002 Aug;87(8):3902-10.

274. Rausch ME, Weisberg S, Vardhana P, Tortoriello DV. Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. Int J Obes (Lond). 2008 Mar;32(3):451-63.

275. Ye J, Gao Z, Yin J, He Q. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. Am J Physiol Endocrinol Metab. 2007 Oct;293(4):E1118-28.

276. Hodson L, Humphreys SM, Karpe F, Frayn KN. Metabolic signatures of human adipose tissue hypoxia in obesity. Diabetes. 2013 May;62(5):1417-25.

277. Klimcakova E, Roussel B, Marquez-Quinones A, Kovacova Z, Kovacikova M, Combes M, et al. Worsening of obesity and metabolic status yields similar molecular adaptations in human subcutaneous and

visceral adipose tissue: decreased metabolism and increased immune response. J Clin Endocrinol Metab. 2011 Jan;96(1):E73-82.

278. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. 1995 Jan;1(1):27-31.

279. Cao Y. Adipose tissue angiogenesis as a therapeutic target for obesity and metabolic diseases. Nat Rev Drug Discov. 2010 Feb;9(2):107-15.

280. Cao Y. Angiogenesis modulates adipogenesis and obesity. J Clin Invest. 2007 Sep;117(9):2362-8.

281. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006 Dec 14;444(7121):860-7.

282. Bakker W, Eringa EC, Sipkema P, van Hinsbergh VWM. Endothelial dysfunction and diabetes: roles of hyperglycemia, impaired insulin signaling and obesity. Cell Tissue Res. 2009 Jan;335(1):165-89.

283. Jansson P. Endothelial dysfunction in insulin resistance and type 2 diabetes. J Intern Med. 2007 Aug;262(2):173-83.

284. Xue Y, Petrovic N, Cao R, Larsson O, Lim S, Chen S, et al. Hypoxia-independent angiogenesis in adipose tissues during cold acclimation. Cell Metab. 2009 Jan 7;9(1):99-109.

285. Kubli DA, Gustafsson AB. Mitochondria and mitophagy: the yin and yang of cell death control. Circ Res. 2012 Oct 12;111(9):1208-21.

286. Kovsan J, Bluher M, Tarnovscki T, Kloting N, Kirshtein B, Madar L, et al. Altered autophagy in human adipose tissues in obesity. J Clin Endocrinol Metab. 2011 Feb;96(2):E268-77.

287. Ost A, Svensson K, Ruishalme I, Brannmark C, Franck N, Krook H, et al. Attenuated mTOR signaling and enhanced autophagy in adipocytes from obese patients with type 2 diabetes. Mol Med. 2010 Jul-Aug;16(7-8):235-46.

288. Fischer-Posovszky P, Wang QA, Asterholm IW, Rutkowski JM, Scherer PE. Targeted deletion of adipocytes by apoptosis leads to adipose tissue recruitment of alternatively activated M2 macrophages. Endocrinology. 2011 Aug;152(8):3074-81.

289. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. J Clin Invest. 2011 Jun;121(6):2111-7.

290. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008 Jul 24;454(7203):428-35.

291. Hauner H. Secretory factors from human adipose tissue and their functional role. Proc Nutr Soc. 2005 May;64(2):163-9.

292. Clowes GHJ, Martin H, Walji S, Hirsch E, Gazitua R, Goodfellow R. Blood insulin responses to blood glucose levels in high output sepsis and spetic shock. Am J Surg. 1978 Apr;135(4):577-83.

293. Apostolopoulos V, de Courten MPJ, Stojanovska L, Blatch GL, Tangalakis K, de Courten B. The complex immunological and inflammatory network of adipose tissue in obesity. Mol Nutr Food Res. 2016 Jan;60(1):43-57.

294. Ruan H, Lodish HF. Regulation of insulin sensitivity by adipose tissue-derived hormones and inflammatory cytokines. Curr Opin Lipidol. 2004 Jun;15(3):297-302.

295. Schipper HS, Prakken B, Kalkhoven E, Boes M. Adipose tissue-resident immune cells: key players in immunometabolism. Trends Endocrinol Metab. 2012 Aug;23(8):407-15.

296. Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, et al. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes. 2005 Aug;54(8):2277-86.

297. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nature Rev Immunol. 2011 Feb;11(2):85-97.

298. Yang R, Barouch LA. Leptin signaling and obesity: cardiovascular consequences. Circ Res. 2007 Sep 14;101(6):545-59.

299. Permana PA, Menge C, Reaven PD. Macrophage-secreted factors induce adipocyte inflammation and insulin resistance. Biochem Biophys Res Commun. 2006 Mar 10;341(2):507-14.

300. Suganami T, Nishida J, Ogawa Y. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. Arterioscler Thromb Vasc Biol. 2005 Oct;25(10):2062-8.

301. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993 Jan 1;259(5091):87-91.

302. Rosen ED, Spiegelman BM. Adipocytes as regulators of energy balance and glucose homeostasis. Nature. 2006 Dec 14;444(7121):847-53.

303. Mraz M, Haluzik M. The role of adipose tissue immune cells in obesity and low-grade inflammation. J Endocrinol. 2014 Sep;222(3):R113-27.

304. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature. 1997 Oct 9;389(6651):610-4.

305. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science. 1996 Feb 2;271(5249):665-8.

306. Hauner H, Petruschke T, Russ M, Rohrig K, Eckel J. Effects of tumour necrosis factor alpha (TNF alpha) on glucose transport and lipid metabolism of newly-differentiated human fat cells in cell culture. Diabetologia. 1995 Jul;38(7):764-71.

307. Stephens JM, Pekala PH. Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 adipocytes by tumor necrosis factor-alpha. J Biol Chem. 1991 Nov 15;266(32):21839-45.

308. Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol. 2008 May;9(5):367-77.

309. Ruan H, Pownall HJ, Lodish HF. Troglitazone antagonizes tumor necrosis factor-alpha-induced reprogramming of adipocyte gene expression by inhibiting the transcriptional regulatory functions of NF-kappaB. J Biol Chem. 2003 Jul 25;278(30):28181-92.

310. Fain JN. Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. Vitam Horm. 2006;74:443-77.

311. Lehr S, Hartwig S, Sell H. Adipokines: a treasure trove for the discovery of biomarkers for metabolic disorders. Proteomics Clin Appl. 2012 Jan;6(1-2):91-101.

312. Mlinar B, Marc J, Janez A, Pfeifer M. Molecular mechanisms of insulin resistance and associated diseases. Clin Chim Acta. 2007 Jan;375(1-2):20-35.

313. Freychet P, Roth J, Neville DMJ. Insulin receptors in the liver: specific binding of (125 I)insulin to the plasma membrane and its relation to insulin bioactivity. Proc Natl Acad Sci U S A. 1971 Aug;68(8):1833-7.

314. White MF. IRS proteins and the common path to diabetes. Am J Physiol Endocrinol Metab. 2002 Sep;283(3):E413-22.

315. Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G. Relationship between degree of obesity and in vivo insulin action in man. Am J Physiol. 1985 Mar;248(3 Pt 1):E286-91.

316. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med. 2001 Sep 13;345(11):790-7.

317. DeFronzo R, Deibert D, Hendler R, Felig P, Soman V. Insulin sensitivity and insulin binding to monocytes in maturity-onset diabetes. J Clin Invest. 1979 May;63(5):939-46.

318. Forouhi NG, Jenkinson G, Thomas EL, Mullick S, Mierisova S, Bhonsle U, et al. Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. Diabetologia. 1999 Aug;42(8):932-5.

319. Banerji MA, Buckley MC, Chaiken RL, Gordon D, Lebovitz HE, Kral JG. Liver fat, serum triglycerides and visceral adipose tissue in insulin-sensitive and insulin-resistant black men with NIDDM. Int J Obes Relat Metab Disord. 1995 Dec;19(12):846-50.

320. Goto T, Onuma T, Takebe K, Kral JG. The influence of fatty liver on insulin clearance and insulin resistance in non-diabetic Japanese subjects. Int J Obes Relat Metab Disord. 1995 Dec;19(12):841-5.

321. Koyama K, Chen G, Lee Y, Unger RH. Tissue triglycerides, insulin resistance, and insulin production: implications for hyperinsulinemia of obesity. Am J Physiol. 1997 Oct;273(4 Pt 1):E708-13.

322. Assimacopoulos-Jeannet F. Fat storage in pancreas and in insulin-sensitive tissues in pathogenesis of type 2 diabetes. Int J Obes Relat Metab Disord. 2004 Dec;28(Suppl 4):S53-7.

323. Tabak AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimaki M, Witte DR. Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. Lancet. 2009 Jun 27;373(9682):2215-21.

324. Sicree RA, Zimmet PZ, King HO, Coventry JS. Plasma insulin response among Nauruans. Prediction of deterioration in glucose tolerance over 6 yr. Diabetes. 1987 Feb;36(2):179-86.

325. Birkenfeld AL, Shulman GI. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology. 2014 Feb;59(2):713-23.

326. Seppala-Lindroos A, Vehkavaara S, Hakkinen A, Goto T, Westerbacka J, Sovijarvi A, et al. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endocrinol Metab. 2002 Jul;87(7):3023-8.

327. RANDLE PJ, GARLAND PB, HALES CN, NEWSHOLME EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet. 1963 Apr 13;1(7285):785-9.

328. Savage DB, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. Physiol Rev. 2007 Apr;87(2):507-20.

329. Boden G, Jadali F, White J, Liang Y, Mozzoli M, Chen X, et al. Effects of fat on insulin-stimulated carbohydrate metabolism in normal men. J Clin Invest. 1991 Sep;88(3):960-6.

330. Boden G, Chen X, Ruiz J, White JV, Rossetti L. Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest. 1994 Jun;93(6):2438-46.

331. Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. Diabetes. 2002 Jul;51(7):2005-11.

332. Coppack SW, Evans RD, Fisher RM, Frayn KN, Gibbons GF, Humphreys SM, et al. Adipose tissue metabolism in obesity: lipase action in vivo before and after a mixed meal. Metabolism. 1992 Mar;41(3):264-72.

333. Carpentier A, Mittelman SD, Lamarche B, Bergman RN, Giacca A, Lewis GF. Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. Am J Physiol. 1999 Jun;276(6 Pt 1):E1055-66.

334. Grill V, Qvigstad E. Fatty acids and insulin secretion. Br J Nutr. 2000 Mar;83(Suppl 1):S79-84.

335. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, et al. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature. 2004 Sep 9;431(7005):200-5.

336. Elghazi L, Balcazar N, Blandino-Rosano M, Cras-Meneur C, Fatrai S, Gould AP, et al. Decreased IRS signaling impairs beta-cell cycle progression and survival in transgenic mice overexpressing S6K in beta-cells. Diabetes. 2010 Oct;59(10):2390-9.

337. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol. 2011;29:415-45.

338. Rakatzi I, Mueller H, Ritzeler O, Tennagels N, Eckel J. Adiponectin counteracts cytokine- and fatty acid-induced apoptosis in the pancreatic beta-cell line INS-1. Diabetologia. 2004 Feb;47(2):249-58.

339. Arner P. Free fatty acids--do they play a central role in type 2 diabetes? Diabetes Obes Metab. 2001 Aug;3(Suppl 1):S11-9.

340. Tan CY, Vidal-Puig A. Adipose tissue expandability: the metabolic problems of obesity may arise from the inability to become more obese. Biochem Soc Trans. 2008 Oct;36(Pt 5):935-40.

341. Abate N, Garg A, Peshock RM, Stray-Gundersen J, Grundy SM. Relationships of generalized and regional adiposity to insulin sensitivity in men. J Clin Invest. 1995 Jul;96(1):88-98.

342. Abate N, Garg A, Peshock RM, Stray-Gundersen J, Adams-Huet B, Grundy SM. Relationship of generalized and regional adiposity to insulin sensitivity in men with NIDDM. Diabetes. 1996 Dec;45(12):1684-93.

343. Goodpaster BH, Thaete FL, Simoneau JA, Kelley DE. Subcutaneous abdominal fat and thigh muscle composition predict insulin sensitivity independently of visceral fat. Diabetes. 1997 Oct;46(10):1579-85.

344. Ferreira I, Henry RMA, Twisk JWR, van Mechelen W, Kemper HCG, Stehouwer CDA, et al. The metabolic syndrome, cardiopulmonary fitness, and subcutaneous trunk fat as independent determinants of arterial stiffness: the Amsterdam Growth and Health Longitudinal Study. Arch Intern Med. 2005 Apr 25;165(8):875-82.

345. Demerath EW, Reed D, Rogers N, Sun SS, Lee M, Choh AC, et al. Visceral adiposity and its anatomical distribution as predictors of the metabolic syndrome and cardiometabolic risk factor levels. Am J Clin Nutr. 2008 Nov;88(5):1263-71.

346. Klein S, Allison DB, Heymsfield SB, Kelley DE, Leibel RL, Nonas C, et al. Waist circumference and cardiometabolic risk: a consensus statement from shaping America's health: Association for Weight Management and Obesity Prevention; NAASO, the Obesity Society; the American Society for Nutrition; and the American Diabetes Association. Diabetes Care. 2007 Jun;30(6):1647-52.

347. Garg A. Regional adiposity and insulin resistance. J Clin Endocrinol Metab. 2004 Sep;89(9):4206-10.

348. Kabir M, Catalano KJ, Ananthnarayan S, Kim SP, Van Citters GW, Dea MK, et al. Molecular evidence supporting the portal theory: a causative link between visceral adiposity and hepatic insulin resistance. Am J Physiol Endocrinol Metab. 2005 Feb;288(2):E454-61.

349. Jensen MD, Johnson CM. Contribution of leg and splanchnic free fatty acid (FFA) kinetics to postabsorptive FFA flux in men and women. Metabolism. 1996 May;45(5):662-6.

350. Jennings CL, Lambert EV, Collins M, Joffe Y, Levitt NS, Goedecke JH. Determinants of insulin-resistant phenotypes in normal-weight and obese Black African women. Obesity (Silver Spring). 2008 Jul;16(7):1602-9.

351. Fabbrini E, Tamboli RA, Magkos F, Marks-Shulman PA, Eckhauser AW, Richards WO, et al. Surgical removal of omental fat does not improve insulin sensitivity and cardiovascular risk factors in obese adults. Gastroenterology. 2010 Aug;139(2):448-55.

352. Poussin C, Hall D, Minehira K, Galzin A, Tarussio D, Thorens B. Different transcriptional control of metabolism and extracellular matrix in visceral and subcutaneous fat of obese and rimonabant treated mice. PLoS ONE. 2008;3(10):e3385.

353. Linder K, Arner P, Flores-Morales A, Tollet-Egnell P, Norstedt G. Differentially expressed genes in visceral or subcutaneous adipose tissue of obese men and women. J Lipid Res. 2004 Jan;45(1):148-54.

354. Gesta S, Bluher M, Yamamoto Y, Norris AW, Berndt J, Kralisch S, et al. Evidence for a role of developmental genes in the origin of obesity and body fat distribution. Proc Natl Acad Sci U S A. 2006 Apr 25;103(17):6676-81.

355. van Harmelen V, Dicker A, Ryden M, Hauner H, Lonnqvist F, Naslund E, et al. Increased lipolysis and decreased leptin production by human omental as compared with subcutaneous preadipocytes. Diabetes. 2002 Jul;51(7):2029-36.

356. Arner P. Not all fat is alike. Lancet. 1998 May 2;351(9112):1301-2.

357. Ramis JM, Franssen-van Hal NLW, Kramer E, Llado I, Bouillaud F, Palou A, et al. Carboxypeptidase E and thrombospondin-1 are differently expressed in subcutaneous and visceral fat of obese subjects. Cell Mol Life Sci. 2002 Nov;59(11):1960-71.

358. Dusserre E, Moulin P, Vidal H. Differences in mRNA expression of the proteins secreted by the adipocytes in human subcutaneous and visceral adipose tissues. Biochim Biophys Acta. 2000 Jan 3;1500(1):88-96.

359. Gabrielsson BG, Johansson JM, Lonn M, Jernas M, Olbers T, Peltonen M, et al. High expression of complement components in omental adipose tissue in obese men. Obes Res. 2003 Jun;11(6):699-708.

360. Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, et al. A transgenic model of visceral obesity and the metabolic syndrome. Science. 2001 Dec 7;294(5549):2166-70.

361. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes. 2007 Apr;56(4):1010-3.

362. Virtue S, Vidal-Puig A. Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome--an allostatic perspective. Biochim Biophys Acta. 2010 Mar;1801(3):338-49.

363. Schaffer JE. Lipotoxicity: when tissues overeat. Curr Opin Lipidol. 2003 Jun;14(3):281-7.

364. Lingvay I, Esser V, Legendre JL, Price AL, Wertz KM, Adams-Huet B, et al. Noninvasive quantification of pancreatic fat in humans. J Clin Endocrinol Metab. 2009 Oct;94(10):4070-6.

365. McGavock JM, Lingvay I, Zib I, Tillery T, Salas N, Unger R, et al. Cardiac steatosis in diabetes mellitus: a 1H-magnetic resonance spectroscopy study. Circulation. 2007 Sep 4;116(10):1170-5.

366. Sims EA. Are there persons who are obese, but metabolically healthy? Metabolism. 2001 Dec;50(12):1499-504.

367. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes. 1988 Dec;37(12):1595-607.

368. Capeau J, Magre J, Lascols O, Caron M, Bereziat V, Vigouroux C, et al. Diseases of adipose tissue: genetic and acquired lipodystrophies. Biochem Soc Trans. 2005 Nov;33(Pt 5):1073-7.

369. Rui L. Energy metabolism in the liver. Compr Physiol. 2014 Jan;4(1):177-97.

370. Ekberg K, Landau BR, Wajngot A, Chandramouli V, Efendic S, Brunengraber H, et al. Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. Diabetes. 1999 Feb;48(2):292-8.

371. Magnusson I, Rothman DL, Gerard DP, Katz LD, Shulman GI. Contribution of hepatic glycogenolysis to glucose production in humans in response to a physiological increase in plasma glucagon concentration. Diabetes. 1995 Feb;44(2):185-9.

372. Tessari P, Coracina A, Cosma A, Tiengo A. Hepatic lipid metabolism and non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis. 2009 May;19(4):291-302.

373. Frayn KN, Arner P, Yki-Jarvinen H. Fatty acid metabolism in adipose tissue, muscle and liver in health and disease. Essays Biochem. 2006;42:89-103.

374. Rizza RA, Mandarino LJ, Gerich JE. Dose-response characteristics for effects of insulin on production and utilization of glucose in man. Am J Physiol. 1981 Jun;240(6):E630-9.

375. Singhal P, Caumo A, Carey PE, Cobelli C, Taylor R. Regulation of endogenous glucose production after a mixed meal in type 2 diabetes. Am J Physiol Endocrinol Metab. 2002 Aug;283(2):E275-83.

376. Acheson KJ, Schutz Y, Bessard T, Anantharaman K, Flatt JP, Jequier E. Glycogen storage capacity and de novo lipogenesis during massive carbohydrate overfeeding in man. Am J Clin Nutr. 1988 Aug;48(2):240-7.

377. Ravikumar B, Carey PE, Snaar JEM, Deelchand DK, Cook DB, Neely RDG, et al. Real-time assessment of postprandial fat storage in liver and skeletal muscle in health and type 2 diabetes. Am J Physiol Endocrinol Metab. 2005 Apr;288(4):E789-97.

378. Wasastjerna C, Reissell P, Karjalainen J, Ekelund P. Fatty liver in diabetes. A cytological study. Acta Med Scand. 1972 Mar;191(3):225-8.

379. Yki-Jarvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol. 2014 Nov;2(11):901-10.

380. Kechagias S, Ernersson A, Dahlqvist O, Lundberg P, Lindstrom T, Nystrom FH, et al. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. Gut. 2008 May;57(5):649-54.

381. Luyckx FH, Lefebvre PJ, Scheen AJ. Non-alcoholic steatohepatitis: association with obesity and insulin resistance, and influence of weight loss. Diabetes Metab. 2000 Apr;26(2):98-106.

382. Yki-Jarvinen H. Fat in the liver and insulin resistance. Ann Med. 2005;37(5):347-56.

383. Pietilainen KH, Rissanen A, Kaprio J, Makimattila S, Hakkinen AM, Westerbacka J, et al. Acquired obesity is associated with increased liver fat, intra-abdominal fat, and insulin resistance in young adult monozygotic twins. Am J Physiol Endocrinol Metab. 2005 Apr;288(4):E768-74.

384. Kamada Y, Takehara T, Hayashi N. Adipocytokines and liver disease. J Gastroenterol. 2008;43(11):811-22.

385. Ozes ON, Akca H, Mayo LD, Gustin JA, Maehama T, Dixon JE, et al. A phosphatidylinositol 3-kinase/Akt/mTOR pathway mediates and PTEN antagonizes tumor necrosis factor inhibition of insulin signaling through insulin receptor substrate-1. Proc Natl Acad Sci U S A. 2001 Apr 10;98(8):4640-5.

386. Dowman JK, Tomlinson JW, Newsome PN. Pathogenesis of non-alcoholic fatty liver disease. QJM. 2010 Feb;103(2):71-83.

387. Hoyumpa AMJ, Greene HL, Dunn GD, Schenker S. Fatty liver: biochemical and clinical considerations. Am J Dig Dis. 1975 Dec;20(12):1142-70.

388. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005 Jun;41(6):1313-21.

389. Hubscher SG. Histological assessment of non-alcoholic fatty liver disease. Histopathology. 2006 Nov;49(5):450-65.

390. Fabbrini E, Mohammed BS, Magkos F, Korenblat KM, Patterson BW, Klein S. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. Gastroenterology. 2008 Feb;134(2):424-31.

391. Adiels M, Taskinen M, Packard C, Caslake MJ, Soro-Paavonen A, Westerbacka J, et al. Overproduction of large VLDL particles is driven by increased liver fat content in man. Diabetologia. 2006 Apr;49(4):755-65.

392. Korenblat KM, Fabbrini E, Mohammed BS, Klein S. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. Gastroenterology. 2008 May;134(5):1369-75.

393. Yatsuya H, Nihashi T, Li Y, Hotta Y, Matsushita K, Muramatsu T, et al. Independent association of liver fat accumulation with insulin resistance. Obes Res Clin Pract. 2014 Jul-Aug;8(4):e350-5.

394. Bugianesi E, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, et al. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia. 2005 Apr;48(4):634-42.

395. Vanni E, Bugianesi E, Kotronen A, De Minicis S, Yki-Jarvinen H, Svegliati-Baroni G. From the metabolic syndrome to NAFLD or vice versa? Dig Liver Dis. 2010 May;42(5):320-30.

396. Fabbrini E, Magkos F, Mohammed BS, Pietka T, Abumrad NA, Patterson BW, et al. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. Proc Natl Acad Sci U S A. 2009 Sep 8;106(36):15430-5.

397. D'Adamo E, Cali AMG, Weiss R, Santoro N, Pierpont B, Northrup V, et al. Central role of fatty liver in the pathogenesis of insulin resistance in obese adolescents. Diabetes Care. 2010 Aug;33(8):1817-22.

398. Samuel VT, Liu Z, Qu X, Elder BD, Bilz S, Befroy D, et al. Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. J Biol Chem. 2004 Jul 30;279(31):32345-53.

399. Kolak M, Westerbacka J, Velagapudi VR, Wagsater D, Yetukuri L, Makkonen J, et al. Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity. Diabetes. 2007 Aug;56(8):1960-8.

400. Bedogni G, Gastaldelli A, Tiribelli C, Agosti F, De Col A, Fessehatsion R, et al. Relationship between glucose metabolism and non-alcoholic fatty liver disease severity in morbidly obese women. J Endocrinol Invest. 2014 Aug;37(8):739-44.

401. Siddiqui MS, Cheang KL, Luketic VA, Boyett S, Idowu MO, Patidar K, et al. Nonalcoholic Steatohepatitis (NASH) Is Associated with a Decline in Pancreatic Beta Cell (beta-Cell) Function. Dig Dis Sci. 2015 Aug;60(8):2529-37.

402. Kotronen A, Vehkavaara S, Seppala-Lindroos A, Bergholm R, Yki-Jarvinen H. Effect of liver fat on insulin clearance. Am J Physiol Endocrinol Metab. 2007 Dec;293(6):E1709-15.

403. Minehira K, Young SG, Villanueva CJ, Yetukuri L, Oresic M, Hellerstein MK, et al. Blocking VLDL secretion causes hepatic steatosis but does not affect peripheral lipid stores or insulin sensitivity in mice. J Lipid Res. 2008 Sep;49(9):2038-44.

404. Monetti M, Levin MC, Watt MJ, Sajan MP, Marmor S, Hubbard BK, et al. Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. Cell Metab. 2007 Jul;6(1):69-78.

405. Lakka H, Lakka TA, Tuomilehto J, Salonen JT. Abdominal obesity is associated with increased risk of acute coronary events in men. Eur Heart J. 2002 May;23(9):706-13.

406. Andres R. Effect of obesity on total mortality. Int J Obes. 1980;4(4):381-6.

407. Sims EA. Mechanisms of hypertension in the overweight. Hypertension. 1982 Sep-Oct;4(5 Pt 2):43-9.

408. Samocha-Bonet D, Chisholm DJ, Tonks K, Campbell LV, Greenfield JR. Insulin-sensitive obesity in humans - a 'favorable fat' phenotype? Trends Endocrinol Metab. 2012 Mar;23(3):116-24.

409. Stefan N, Haring H, Hu FB, Schulze MB. Metabolically healthy obesity: epidemiology, mechanisms, and clinical implications. Lancet Diabetes Endocrinol. 2013 Oct;1(2):152-62.

410. Pataky Z, Bobbioni-Harsch E, Golay A. Open questions about metabolically normal obesity. Int J Obes (Lond). 2010 Dec;34(Suppl 2):S18-23.

411. Karelis AD, Rabasa-Lhoret R. Obesity: Can inflammatory status define metabolic health?. Nat Rev Endocrinol. 2013 Dec;9(12):694-5.

412. Esser N, L'homme L, De Roover A, Kohnen L, Scheen AJ, Moutschen M, et al. Obesity phenotype is related to NLRP3 inflammasome activity and immunological profile of visceral adipose tissue. Diabetologia. 2013 Nov;56(11):2487-97.

413. Kramer CK, Zinman B, Retnakaran R. Are metabolically healthy overweight and obesity benign conditions?: A systematic review and meta-analysis. Ann Intern Med. 2013 Dec 3;159(11):758-69.

414. Lackey DE, Burk DH, Ali MR, Mostaedi R, Smith WH, Park J, et al. Contributions of adipose tissue architectural and tensile properties toward defining healthy and unhealthy obesity. Am J Physiol Endocrinol Metab. 2014 Feb;306(3):E233-46.

415. Sun K, Wernstedt Asterholm I, Kusminski CM, Bueno AC, Wang ZV, Pollard JW, et al. Dichotomous effects of VEGF-A on adipose tissue dysfunction. Proc Natl Acad Sci U S A. 2012 Apr 10;109(15):5874-9.

416. Ogorodnikova AD, Khan UI, McGinn AP, Zeb I, Budoff MJ, Harman SM, et al. Ectopic fat and adipokines in metabolically benign overweight/obese women: the Kronos Early Estrogen Prevention Study. Obesity (Silver Spring). 2013 Aug;21(8):1726-33.

417. Appleton SL, Seaborn CJ, Visvanathan R, Hill CL, Gill TK, Taylor AW, et al. Diabetes and cardiovascular disease outcomes in the metabolically healthy obese phenotype: a cohort study. Diabetes Care. 2013 Aug;36(8):2388-94.

418. Chang Y, Ryu S, Suh BS, Yun KE, Kim CW, Cho SI. Impact of BMI on the incidence of metabolic abnormalities in metabolically healthy men. Int J Obes (Lond). 2012 Sep;36(9):1187-94.

419. Soriguer F, Gutierrez-Repiso C, Rubio-Martin E, Garcia-Fuentes E, Almaraz MC, Colomo N, et al. Metabolically healthy but obese, a matter of time? Findings from the prospective Pizarra study. J Clin Endocrinol Metab. 2013 Jun;98(6):2318-25.

420. Boomsma D, Busjahn A, Peltonen L. Classical twin studies and beyond. Nat Rev Genet. 2002 Nov;3(11):872-82.

421. Kaprio J, Pulkkinen L, Rose RJ. Genetic and environmental factors in health-related behaviors: studies on Finnish twins and twin families. Twin Res. 2002 Oct;5(5):366-71.

422. Kaprio J. Twin studies in Finland 2006. Twin Res Hum Genet. 2006 Dec;9(6):772-7.

423. Pietilainen KH, Rissanen A, Laamanen M, Lindholm A, Markkula H, Yki-Jarvinen H, et al. Growth patterns in young adult monozygotic twin pairs discordant and concordant for obesity. Twin Res. 2004 Oct;7(5):421-9.

424. Pietrobelli A, Formica C, Wang Z, Heymsfield SB. Dual-energy X-ray absorptiometry body composition model: review of physical concepts. Am J Physiol. 1996 Dec;271(6 Pt 1):E941-51.

425. Graner M, Seppala-Lindroos A, Rissanen A, Hakkarainen A, Lundbom N, Kaprio J, et al. Epicardial fat, cardiac dimensions, and low-grade inflammation in young adult monozygotic twins discordant for obesity. Am J Cardiol. 2012 May 1;109(9):1295-302.

426. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985 Jul;28(7):412-9.

427. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care. 1999 Sep;22(9):1462-70.

428. Yeckel CW, Weiss R, Dziura J, Taksali SE, Dufour S, Burgert TS, et al. Validation of insulin sensitivity indices from oral glucose tolerance test parameters in obese children and adolescents. J Clin Endocrinol Metab. 2004 Mar;89(3):1096-101.

429. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 2004;5(10):R80.

430. Heinonen S, Saarinen L, Naukkarinen J, Rodriguez A, Fruhbeck G, Hakkarainen A, et al. Adipocyte morphology and implications for metabolic derangements in acquired obesity. Int J Obes (Lond). 2014 Nov;38(11):1423-31.

431. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A. 2003 Aug 5;100(16):9440-5.

432. Laakso M, Hautaniemi S. Integrative platform to translate gene sets to networks. Bioinformatics. 2010 Jul 15;26(14):1802-3.

433. Ovaska K, Laakso M, Haapa-Paananen S, Louhimo R, Chen P, Aittomaki V, et al. Large-scale data integration framework provides a comprehensive view on glioblastoma multiforme. Genome Med. 2010;2(9):65.

434. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Research. 2015;doi: 10.1093/nar/gkv007.

435. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr. 1982 Nov;36(5):936-42.

436. Rao J, Scott A. On chi-squared tests for multiway contingency tables with cell proportions estimated from survey data. Annals of Statistics. 1984;12:46-60.

437. Heinonen S, Buzkova J, Muniandy M, Kaksonen R, Ollikainen M, Ismail K, et al. Impaired Mitochondrial Biogenesis in Adipose Tissue in Acquired Obesity. Diabetes. 2015 Sep;64(9):3135-45.

438. Pietilainen KH, Korkeila M, Bogl LH, Westerterp KR, Yki-Jarvinen H, Kaprio J, et al. Inaccuracies in food and physical activity diaries of obese subjects: complementary evidence from doubly labeled water and co-twin assessments. Int J Obes (Lond). 2010 Mar;34(3):437-45.

439. Despres JP, Bouchard C. Monozygotic twin resemblance in fatness and fat cell lipolysis. Acta Genet Med Gemellol (Roma). 1984;33(3):475-80.

440. Jo J, Gavrilova O, Pack S, Jou W, Mullen S, Sumner AE, et al. Hypertrophy and/or Hyperplasia: Dynamics of Adipose Tissue Growth. PLoS Comput Biol. 2009 Mar;5(3):e1000324.

441. Arner P, Spalding KL. Fat cell turnover in humans. Biochem Biophys Res Commun. 2010 May 21;396(1):101-4.

442. Permana PA, Nair S, Lee YH, Luczy-Bachman G, Vozarova De Courten B, Tataranni PA. Subcutaneous abdominal preadipocyte differentiation in vitro inversely correlates with central obesity. Am J Physiol Endocrinol Metab. 2004 Jun;286(6):E958-62.

443. van Tienen FH, van der Kallen CJ, Lindsey PJ, Wanders RJ, van Greevenbroek MM, Smeets HJ. Preadipocytes of type 2 diabetes subjects display an intrinsic gene expression profile of decreased differentiation capacity. Int J Obes (Lond). 2011 Sep;35(9):1154-64.

444. Su X, Magkos F, Zhou D, Eagon JC, Fabbrini E, Okunade AL, et al. Adipose tissue monomethyl branched-chain fatty acids and insulin sensitivity: Effects of obesity and weight loss. Obesity (Silver Spring). 2015 Feb;23(2):329-34.

445. Vankoningsloo S, De Pauw A, Houbion A, Tejerina S, Demazy C, de Longueville F, et al. CREB activation induced by mitochondrial dysfunction triggers triglyceride accumulation in 3T3-L1 preadipocytes. J Cell Sci. 2006 Apr 1;119(Pt 7):1266-82.

446. Bretscher A, Edwards K, Fehon RG. ERM proteins and merlin: integrators at the cell cortex. Nat Rev Mol Cell Biol. 2002 Aug;3(8):586-99.

447. Fidalgo M, Guerrero A, Fraile M, Iglesias C, Pombo CM, Zalvide J. Adaptor protein cerebral cavernous malformation 3 (CCM3) mediates phosphorylation of the cytoskeletal proteins ezrin/radixin/moesin by mammalian Ste20-4 to protect cells from oxidative stress. J Biol Chem. 2012 Mar 30;287(14):11556-65.

448. Otey CA, Rachlin A, Moza M, Arneman D, Carpen O. The palladin/myotilin/myopalladin family of actin-associated scaffolds. Int Rev Cytol. 2005;246:31-58.

449. Chipuk JE, Green DR. PUMA cooperates with direct activator proteins to promote mitochondrial outer membrane permeabilization and apoptosis. Cell Cycle. 2009 Sep 1;8(17):2692-6.

450. Nordstrom EA, Ryden M, Backlund EC, Dahlman I, Kaaman M, Blomqvist L, et al. A human-specific role of cell death-inducing DFFA (DNA fragmentation factor-alpha)-like effector A (CIDEA) in adipocyte lipolysis and obesity. Diabetes. 2005 Jun;54(6):1726-34.

451. Puri V, Ranjit S, Konda S, Nicoloro SM, Straubhaar J, Chawla A, et al. Cidea is associated with lipid droplets and insulin sensitivity in humans. Proc Natl Acad Sci U S A. 2008 Jun 3;105(22):7833-8.

452. Gao H, Mejhert N, Fretz JA, Arner E, Lorente-Cebrian S, Ehrlund A, et al. Early B cell factor 1 regulates adipocyte morphology and lipolysis in white adipose tissue. Cell Metab. 2014 Jun 3;19(6):981-92.

453. Arner E, Westermark PO, Spalding KL, Britton T, Ryden M, Frisen J, et al. Adipocyte turnover: relevance to human adipose tissue morphology. Diabetes. 2010 Jan;59(1):105-9.

454. Laforest S, Labrecque J, Michaud A, Cianflone K, Tchernof A. Adipocyte size as a determinant of metabolic disease and adipose tissue dysfunction. Crit Rev Clin Lab Sci. 2015;52(6):301-13.

455. Salans LB, Horton ES, Sims EA. Experimental obesity in man: cellular character of the adipose tissue. J Clin Invest. 1971 May;50(5):1005-11.

456. Lagathu C, Christodoulides C, Virtue S, Cawthorn WP, Franzin C, Kimber WA, et al. Dact1, a nutritionally regulated preadipocyte gene, controls adipogenesis by coordinating the Wnt/beta-catenin signaling network. Diabetes. 2009 Mar;58(3):609-19.

457. Eriksson-Hogling D, Andersson D, Bäckdahl J, Hoffstedt J, Rössner S, Thorell A, et al. Adipose tissue morphology predicts improved insulin sensitivity following moderate or pronounced weight loss. Int J Obes (Lond). 2015 Feb 10;doi: 10.1038/ijo.2015.18. [Epub ahead of print].

458. Kotronen A, Seppala-Lindroos A, Bergholm R, Yki-Jarvinen H. Tissue specificity of insulin resistance in humans: fat in the liver rather than muscle is associated with features of the metabolic syndrome. Diabetologia. 2008 Jan;51(1):130-8.

459. Kotronen A, Westerbacka J, Bergholm R, Pietilainen KH, Yki-Jarvinen H. Liver fat in the metabolic syndrome. J Clin Endocrinol Metab. 2007 Sep;92(9):3490-7.

460. Naukkarinen J, Rissanen A, Kaprio J, Pietilainen KH. Causes and consequences of obesity: the contribution of recent twin studies. Int J Obes (Lond). 2012 Aug;36(8):1017-24.

461. Huang-Doran I, Sleigh A, Rochford JJ, O'Rahilly S, Savage DB. Lipodystrophy: metabolic insights from a rare disorder. J Endocrinol. 2010 Dec;207(3):245-55.

462. Alligier M, Gabert L, Meugnier E, Lambert-Porcheron S, Chanseaume E, Pilleul F, et al. Visceral fat accumulation during lipid overfeeding is related to subcutaneous adipose tissue characteristics in healthy men. J Clin Endocrinol Metab. 2013 Feb;98(2):802-10.

463. Hackl H, Burkard TR, Sturn A, Rubio R, Schleiffer A, Tian S, et al. Molecular processes during fat cell development revealed by gene expression profiling and functional annotation. Genome Biol. 2005;6(13):R108.

464. Petaja EM, Sevastianova K, Hakkarainen A, Orho-Melander M, Lundbom N, Yki-Jarvinen H. Adipocyte size is associated with NAFLD independent of obesity, fat distribution, and PNPLA3 genotype. Obesity (Silver Spring). 2013 Jun;21(6):1174-9.

465. Fabbrini E, Yoshino J, Yoshino M, Magkos F, Tiemann Luecking C, Samovski D, et al. Metabolically normal obese people are protected from adverse effects following weight gain. J Clin Invest. 2015 Feb;125(2):787-95.

466. Magkos F, Fabbrini E, Mohammed BS, Patterson BW, Klein S. Increased whole-body adiposity without a concomitant increase in liver fat is not associated with augmented metabolic dysfunction. Obesity (Silver Spring). 2010 Aug;18(8):1510-5.

467. Kotronen A, Yki-Jarvinen H, Sevastianova K, Bergholm R, Hakkarainen A, Pietilainen KH, et al. Comparison of the relative contributions of intra-abdominal and liver fat to components of the metabolic syndrome. Obesity (Silver Spring). 2011 Jan;19(1):23-8.

468. Trachta P, Dostalova I, Haluzikova D, Kasalicky M, Kavalkova P, Drapalova J, et al. Laparoscopic sleeve gastrectomy ameliorates mRNA expression of inflammation-related genes in subcutaneous adipose tissue but not in peripheral monocytes of obese patients. Mol Cell Endocrinol. 2014 Mar 5;383(1-2):96-102.

469. Vernochet C, Damilano F, Mourier A, Bezy O, Mori MA, Smyth G, et al. Adipose tissue mitochondrial dysfunction triggers a lipodystrophic syndrome with insulin resistance, hepatosteatosis, and cardiovascular complications. FASEB J. 2014 Oct;28(10):4408-19.

470. Goldfine AB, Fonseca V, Jablonski KA, Pyle L, Staten MA, Shoelson SE, et al. The effects of salsalate on glycemic control in patients with type 2 diabetes: a randomized trial. Ann Intern Med. 2010 Mar 16;152(6):346-57.

471. Kirkland JL, Hollenberg CH, Gillon WS. Age, anatomic site, and the replication and differentiation of adipocyte precursors. Am J Physiol. 1990 Feb;258(2 Pt 1):206-10.

472. Hauner H, Entenmann G, Wabitsch M, Gaillard D, Ailhaud G, Negrel R, et al. Promoting effect of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium. J Clin Invest. 1989 Nov;84(5):1663-70.

473. Kirkland JL, Hollenberg CH, Gillon WS. Ageing, differentiation, and gene expression in rat epididymal preadipocytes. Biochem Cell Biol. 1993 Nov-Dec;71(11-12):556-61.

474. Bains RK, Wells SE, Flavell DM, Fairhall KM, Strom M, Le Tissier P, et al. Visceral obesity without insulin resistance in late-onset obesity rats. Endocrinology. 2004 Jun;145(6):2666-79.

475. Turner N, Heilbronn LK. Is mitochondrial dysfunction a cause of insulin resistance? Trends Endocrinol Metab. 2008 Nov;19(9):324-30.

476. Gao CL, Zhu C, Zhao YP, Chen XH, Ji CB, Zhang CM, et al. Mitochondrial dysfunction is induced by high levels of glucose and free fatty acids in 3T3-L1 adipocytes. Mol Cell Endocrinol. 2010 May 14;320(1-2):25-33.

477. Enguix N, Pardo R, Gonzalez A, Lopez VM, Simo R, Kralli A, et al. Mice lacking PGC-1beta in adipose tissues reveal a dissociation between mitochondrial dysfunction and insulin resistance. Mol Metab. 2013;2(3):215-26.

478. Lynch CJ, Halle B, Fujii H, Vary TC, Wallin R, Damuni Z, et al. Potential role of leucine metabolism in the leucine-signaling pathway involving mTOR. Am J Physiol Endocrinol Metab. 2003 Oct;285(4):E854-63.

479. Naukkarinen J, Heinonen S, Hakkarainen A, Lundbom J, Vuolteenaho K, Saarinen L, et al. Characterising metabolically healthy obesity in weight-discordant monozygotic twins. Diabetologia. 2014 Jan;57(1):167-76.

480. Chen M, Macpherson A, Owens J, Wittert G, Heilbronn LK. Obesity alone or with type 2 diabetes is associated with tissue specific alterations in DNA methylation and gene expression of PPARGC1A and IGF2. Journal of Diabetes Research and Clinical Metabolism. 2012;1:16 http://dx.doi.org/10.7243/2050-0866-1-16.

481. Cildir G, Akincilar SC, Tergaonkar V. Chronic adipose tissue inflammation: all immune cells on the stage. Trends Mol Med. 2013 Aug;19(8):487-500.

482. Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology. 2004 May;145(5):2273-82.

483. Meijer K, de Vries M, Al-Lahham S, Bruinenberg M, Weening D, Dijkstra M, et al. Human primary adipocytes exhibit immune cell function: adipocytes prime inflammation independent of macrophages. PLoS ONE. 2011;6(3):e17154.

484. Khazen W, M'bika J, Tomkiewicz C, Benelli C, Chany C, Achour A, et al. Expression of macrophage-selective markers in human and rodent adipocytes. FEBS Lett. 2005 Oct 24;579(25):5631-4.

485. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab. 1997 Dec;82(12):4196-200.

486. Bogacka I, Xie H, Bray GA, Smith SR. Pioglitazone induces mitochondrial biogenesis in human subcutaneous adipose tissue in vivo. Diabetes. 2005 May;54(5):1392-9.

487. Asemi Z, Samimi M, Tabassi Z, Shakeri H, Esmaillzadeh A. Vitamin D supplementation affects serum high-sensitivity C-reactive protein, insulin resistance, and biomarkers of oxidative stress in pregnant women. J Nutr. 2013 Sep;143(9):1432-8.

488. Fang F, Kang Z, Wong C. Vitamin E tocotrienols improve insulin sensitivity through activating peroxisome proliferator-activated receptors. Mol Nutr Food Res. 2010 Mar;54(3):345-52.

489. Fridlyand LE, Philipson LH. Reactive species, cellular repair and risk factors in the onset of type 2 diabetes mellitus: review and hypothesis. Curr Diabetes Rev. 2006 May;2(2):241-59.

490. Shen W, Liu K, Tian C, Yang L, Li X, Ren J, et al. R-alpha-lipoic acid and acetyl-L-carnitine complementarily promote mitochondrial biogenesis in murine 3T3-L1 adipocytes. Diabetologia. 2008 Jan;51(1):165-74.

491. de Oliveira AM, Rondo PHC, Luzia LA, D'Abronzo FH, Illison VK. The effects of lipoic acid and alpha-tocopherol supplementation on the lipid profile and insulin sensitivity of patients with type 2 diabetes mellitus: a randomized, double-blind, placebo-controlled trial. Diabetes Res Clin Pract. 2011 May;92(2):253-60.

492. Czernichow S, Vergnaud A, Galan P, Arnaud J, Favier A, Faure H, et al. Effects of long-term antioxidant supplementation and association of serum antioxidant concentrations with risk of metabolic syndrome in adults. Am J Clin Nutr. 2009 Aug;90(2):329-35.

493. Clapham JC, Arch JRS. Targeting thermogenesis and related pathways in anti-obesity drug discovery. Pharmacol Ther. 2011 Sep;131(3):295-308.

494. Harper JA, Dickinson K, Brand MD. Mitochondrial uncoupling as a target for drug development for the treatment of obesity. Obes Rev. 2001 Nov;2(4):255-65.

495. Hsiao AL, Santucci KA, Seo-Mayer P, Mariappan MR, Hodsdon ME, Banasiak KJ, et al. Pediatric fatality following ingestion of dinitrophenol: postmortem identification of a "dietary supplement". Clin Toxicol (Phila). 2005;43(4):281-5.

496. Kitada M, Kume S, Takeda-Watanabe A, Tsuda S, Kanasaki K, Koya D. Calorie restriction in overweight males ameliorates obesity-related metabolic alterations and cellular adaptations through anti-aging effects, possibly including AMPK and SIRT1 activation. Biochim Biophys Acta. 2013 Oct;1830(10):4820-7.

497. Schenk S, McCurdy CE, Philp A, Chen MZ, Holliday MJ, Bandyopadhyay GK, et al. Sirt1 enhances skeletal muscle insulin sensitivity in mice during caloric restriction. J Clin Invest. 2011 Nov;121(11):4281-8.

498. Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, et al. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. Nature. 2007 Nov 29;450(7170):712-6.

499. Mercken EM, Mitchell SJ, Martin-Montalvo A, Minor RK, Almeida M, Gomes AP, et al. SRT2104 extends survival of male mice on a standard diet and preserves bone and muscle mass. Aging Cell. 2014 Oct;13(5):787-96.

500. Arkan MC, Hevener AL, Greten FR, Maeda S, Li Z, Long JM, et al. IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med. 2005 Feb;11(2):191-8.

501. Reilly SM, Chiang S, Decker SJ, Chang L, Uhm M, Larsen MJ, et al. An inhibitor of the protein kinases TBK1 and IKK-e improves obesity-related metabolic dysfunctions in mice. Nat Med. 2013 Mar;19(3):313-21.

502. Reilly SM, Ahmadian M, Zamarron BF, Chang L, Uhm M, Poirier B, et al. A subcutaneous adipose tissue-liver signalling axis controls hepatic gluconeogenesis. Nat Commun. 2015;6:6047.

503. Dominguez H, Storgaard H, Rask-Madsen C, Steffen Hermann T, Ihlemann N, Baunbjerg Nielsen D, et al. Metabolic and vascular effects of tumor necrosis factor-alpha blockade with etanercept in obese patients with type 2 diabetes. J Vasc Res. 2005 Nov-Dec;42(6):517-25.

504. Sullivan TJ, Miao Z, Zhao BN, Ertl LS, Wang Y, Krasinski A, et al. Experimental evidence for the use of CCR2 antagonists in the treatment of type 2 diabetes. Metabolism. 2013 Nov;62(11):1623-32.

505. Li P, Oh DY, Bandyopadhyay G, Lagakos WS, Talukdar S, Osborn O, et al. LTB4 promotes insulin resistance in obese mice by acting on macrophages, hepatocytes and myocytes. Nat Med. 2015 Mar;21(3):239-47.

506. Liston TE, Conklyn MJ, Houser J, Wilner KD, Johnson A, Apseloff G, et al. Pharmacokinetics and pharmacodynamics of the leukotriene B4 receptor antagonist CP-105,696 in man following single oral administration. Br J Clin Pharmacol. 1998 Feb;45(2):115-21.

507. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev. 2004 Jan;84(1):277-359.

508. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang A, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell. 2012 Jul 20;150(2):366-76.

509. Sun K, Halberg N, Khan M, Magalang UJ, Scherer PE. Selective inhibition of hypoxia-inducible factor 1alpha ameliorates adipose tissue dysfunction. Mol Cell Biol. 2013 Mar;33(5):904-17.

510. Rupnick MA, Panigrahy D, Zhang C, Dallabrida SM, Lowell BB, Langer R, et al. Adipose tissue mass can be regulated through the vasculature. Proc Natl Acad Sci U S A. 2002 Aug 6;99(16):10730-5.

511. Brakenhielm E, Cao R, Gao B, Angelin B, Cannon B, Parini P, et al. Angiogenesis inhibitor, TNP-470, prevents diet-induced and genetic obesity in mice. Circ Res. 2004 Jun 25;94(12):1579-88.

512. Tam J, Duda DG, Perentes JY, Quadri RS, Fukumura D, Jain RK. Blockade of VEGFR2 and not VEGFR1 can limit diet-induced fat tissue expansion: role of local versus bone marrow-derived endothelial cells. PLoS ONE. 2009;4(3):e4974.

Recent Publications in this Series

53/2016 Vasili Stegajev
Histamine Receptor 4 (H4R) In pathogenesis of Sjögren's Syndrome
54/2016 Katriina Viitasalo
Shift Work and Cardiovascular Risk Factors — Prevention among Airline Employees
55/2016 Michal Stepniewski
Computational Studies on Membrane Proteins and Membrane-Drug Interactions
56/2016 Jaana Oikkonen
Genetics and Genomics of Musical Abilities
57/2016 Hanna Antila
Activation of the TrkB Neurotrophin Receptor by Antidepressant Drugs
58/2016 Elina Rydman
Inflammatory Effects of Nanosized Titanium Dioxide and Carbon Nanotube Pulmonary Exposure
59/2016 Satu Mäki-Nevala
Molecular Markers in Finnish Lung Cancers
60/2016 Mari H. Tervaniemi
Gene Expression and Functional Studies on Psoriatic Epidermis
61/2016 Kristina Malinovskaja-Gomez
Transdermal Iontophoresis – Delivery Control by Ion-Exchange Fibers and Nanocarriers
62/2016 Wojciech Cypryk
Extracellular Vesicles in Innate Immunity - Proteomic Investigations
63/2016 Lauri Vaahtera
Apoplastic ROS and Transcriptional Response in Plant Stress Signaling
64/2016 Hannele Poutiainen
Mikä herättää terveydenhoitajan huolen? Huolen tunnistamisen ja toimimisen haasteet lastenneuvolassa ja kouluterveydenhuollossa
65/2016 Jenni Viinamäki
Analysis of Fatal Poisonings Due to Toxic Alcohols and Drugs — Focus on Metabolites
66/2016 Sari Riihijärvi
Host- and Tumour-related Prognostic Factors in Diffuse Large B-cell Lymphoma
67/2016 Tatu Lajunen
Liposomal Drug Delivery: Light Triggered Drug Release and Targeting to the Posterior Segment of the Eye
68/2016 Kristian Taipale
Immunologic Effects of Cancer Therapy with Oncolytic Adenoviruses
69/2016 Maarit Dimitrow
Development and Validation of a Drug-related Problem Risk Assessment Tool for Use by Practical Nurses Working With Community-Dwelling Aged
70/2016 Vilma Aho
Kuolema kuittaa univelat? Effects of Cumulative Sleep Loss on Immune Functions and Lipid Metabolism
71/2016 Aino Salminen
Matrix Metalloproteinase 8: Genetic, Diagnostic, and Therapeutic Approaches
72/2016 Maili Jakobson
Molecular Mechanisms Controlling Neuronal Bak Expression
73/2016 Lukasz Kuryk
Strategies to Enhance Efficacy of Oncolytic Virotherapy

Helsinki 2016 ISSN 2342-3161 ISBN 978-951-51-2364-0